LANDSCAPE-LEVEL INFLUENCES ON COMMUNITY COMPOSITION AND ECOSYSTEM FUNCTION IN A LARGE RIVER ECOSYSTEM

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LANDSCAPE-LEVEL INFLUENCES ON COMMUNITY COMPOSITION AND ECOSYSTEM FUNCTION IN A LARGE RIVER ECOSYSTEM

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DEDICATION

This dissertation is dedicated to my grandparents, William Louis Becker (1918 – 1999) and Dorothy Alice Becker (1914-2000). From them I learned the importance honesty, hard work, to not fear the unknown, and doing the right thing, even when it is not the easy thing. I also learned the value of education and of laughter. We should never stop learning and never take ourselves so seriously that we stop being able to laugh. Their unending desire to make the world a better place and their relentless optimism in everyone who came after them is sorely missed. I hope that I can do my part.

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ABSTRACT

LANDSCAPE-LEVEL INFLUENCES ON COMMUNITY COMPOSITION AND ECOSYSTEM FUNCTION IN A LARGE RIVER ECOSYSTEM

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Riverine ecosystems are a vitally important link between terrestrial and aquatic ecosystems. Rivers are sites of major biogeochemical processes involved with the carbon (C), nitrogen (N), and phosphorous (P) cycles, providing critically important ecosystem services, and providing habitat for numerous groups of aquatic taxa. Although riverine systems have been a core component in human cultural and economic development, they have long served as a dumping ground for wastes and undesirable substances. Additionally, landscape development by humans has often happened without an understanding of the impact on riverine systems, and the ecological integrity of many river systems is increasingly threatened. However, critical gaps exist in our knowledge about river-system ecology and ecological function, and their link to terrestrial landscapes.

To better understand the ecology of riverine ecosystems, researchers need to study them in the context of the larger landscape. In-stream aquatic nutrients are influenced by a multitude of factors, both internal and external to the aquatic realm. Traditionally, much of the impact assessment has centered on the evaluation of land-use patterns in a catchment. However, land-use patterns are not independent of the physiographic context of a system (e.g. climate, topography, geology). Very few studies have attempted to parse out the independent influences of land-use versus physiographic context. Determining the independent effects of land-use and physiographic conditions, and at which scale they should be assessed (e.g., local, riparian, or watershed), has implications for monitoring and restoration programs. In Chapter 1 of this dissertation I report my investigation on the influence of varying scales of land use assessment and physiographic environmental gradients on aquatic nutrient dynamics. Also, I provide the first explicit assessment of the different influences of land-use and physiographic context on nutrients.

Understanding how different taxa in communities interact and how organisms are influenced by and interact with their environment is the central goal of the study of ecology. Therefore, it is important to focus research not only on how biological communities respond to changes in land use, water quality, or environmental gradients, but how the abundance of one taxonomic group responds to changes in the abundance of other taxonomic groups in the community. In addition to responding to environmental conditions, communities can be structured by predator-prey dynamics, competitive interactions and niche partitioning, as well as differing dispersal ability. Additionally, there is current controversy about whether community interactions or environmental conditions structure the spatial patterns of communities on a landscape. It is important to properly interpret which mechanisms are structuring biotic communities if we are to adapt conservation and management efforts to different scenarios of climate change or human alteration

of the landscape. In Chapter 2 of this dissertation I integrated the physicochemical data from Chapter 1 with invertebrate and fish community data to investigate biogeographical patterns of community concordance in the Brazos River watershed, and the interactions with environmental and spatial gradients.

Bacteria are one of the most abundant and diverse forms of life on the planet. They are involved in and essential to nearly every biogeochemical cycle, including C processing and the cycles for N, P, and sulfur (S). Bacteria are also responsible for processing large amounts of non-living organic C and nutrients into forms that can be used by higher trophic level organisms. The relationship between bacterial production (the use of carbon for new tissue), bacterial respiration (the use of carbon for metabolism and cell maintenance), and total carbon consumption in riverine systems is relatively understudied. Terrestrially derived carbon is an important subsidy to many aquatic systems and bacterial production is often related to organic matter concentrations. Additionally, the interactions that bacterial community composition has with measures of function and nutrient conditions are also relatively understudied. Although advances in the understanding of bacterial ecology have been made, elucidating the specific role that bacterial community composition has in mediating ecosystem function remains a challenge. Recent developments in the areas of bioinformatics have greatly improved the detail at which we examine microbial communities, and are changing our understanding of environmental factors that drive patterns of biogeography in microbial communities. In Chapter 3 of this dissertation, I investigated landscape-level patterns of bacterial ecosystem function and bacterial community composition, and related both to nutrient and environmental conditions.

Using the Brazos River (TX) as my study system, the research presented in this dissertation addressed these gaps as they relate to nutrient cycling, community composition of major groups of biota (fish, invertebrate, and bacterial communities),

and the function of bacteria in riverine systems. I found that nutrient conditions in large riverine systems are largely influenced by landscape-scale environmental gradients, with land-use/land-cover being a secondary influence. The landscape-influenced patterns of environmental conditions were additionally correlated with patterns of species distribution in macroinvertebrate, fish, and bacterial communities. However, there was little evidence that the widespread use of surrogate species in monitoring and restoration plans was justified, as the predictive ability between macroinvertebrates and fish was low. Finally, the patterns of carbon use by bacteria were very different that what has been found in other systems. Both production and respiration appeared to be supported largely by autochthonous production if organic matter. Together, this series of studies highlights the importance of considering both environmental controls and community interactions when assessing large-scale patterns of nutrients, community structure, or ecosystem function.

CHAPTER I

INFLUENCE OF LAND USE AND PHYSIOGRAPHIC GRADIENTS ON NUTRIENTS IN A GULF SLOPE (USA) RIVER SYSTEM[1]

Abstract

Riverine ecosystems are inextricably linked to their watersheds and it is increasingly understood that both land use and physiographic environmental conditions have a large influence on nutrient dynamics and water quality. In order to examine the interactions between land use and physiography and their combined influences on riverine nutrient dynamics, we assessed aquatic nutrients and their relationship with land use and physiographic conditions at multiple spatial scales in the Brazos River (TX, USA), a large complex drainage that spans several ecoregions. Although spatial patterns in physiography and land use were highly correlated, we found that physiographic gradients explained approximately double the amount of the variability in riverine nutrient concentrations than land use (25% and 12%, respectively). The response of nutrient concentrations to spatial patterns of land use and physiography was dependent on both the specific nutrient and scale of analysis; however, elevated dissolved nutrient concentrations were typically associated with areas of higher rainfall, greater stream density, and more intensive human alteration of the watershed. In contrast, particulate nutrients were more responsive to catchment size and seasonality. Through the use of variance partitioning, we determined that seasonality and the amount of rangeland cover in the local area had the strongest independent effects on the concentrations of particulate nutrients, whereas the specific ecoregion type and the coverage of

[1]Becker, J.C., K.J. Rodibaugh, B.J. Labay, T.H. Bonner, Y. Zhang & W.H. Nowlin. *in revision*. Freshwater Science.

urban land use at the level of the sub-catchment had the strongest independent effects on dissolved nutrients. Our study highlights the importance of incorporating physiographic environmental gradients when studying the interactions between a river and its watershed, especially in large, complex watersheds or watersheds that cross steep environmental gradients.

Introduction

Lotic ecosystems form an interconnected network that link upland terrestrial ecosystems to downstream aquatic regions (Allan 2004, Williamson et al. 2008, Thorp et al. 2010). Rivers are sites of major biogeochemical processes involved with the C, N, and P cycles, transforming and processing nutrients, as well as transporting critical nutrients to downstream ecosystems, providing important ecosystem functions and supporting valuable ecosystem services (e.g., food production; Costanza et al. 1997, Thorp et al. 2010, Trabucchi et al. 2012). Although riverine systems are a core component of human cultural and economic development, they have long served as a dumping ground for waste and undesirable substances (Goldman and Horne 1983, Kalff 2001). Landscape development by humans has historically occurred without considering the subsequent impact on riverine systems, and thus the ecological integrity of many river systems is increasingly threatened (Allan et al. 1997). In the United States, the leading sources of impairment to river systems are usually attributed to agricultural activities in a watershed or hydrologic modification (Strayer et al. 2003, USEPA 2009); however, critical gaps exist in our knowledge about river-system ecology and ecosystem function, and their linkages to terrestrial landscapes. These information gaps hinder our ability to effectively manage and restore these systems (Allan 2004, Hoeinghaus et al. 2007, Williamson et al. 2008). A view integrating aquatic and landscape ecology would allow riverine ecologists to address the roles and influences of

multiple stressors on the ecological integrity of riverine systems (Allan 2004).

The effects of landscape-level patterns of land use and structure on riverine ecosystem function are complex (Allan 2004). The influence of different aspects of land use and land cover (LULC), such as percent agricultural land use in a watershed or presence of riparian buffer strips, may vary in intensity for different riverine ecosystem parameters (e.g., in-stream nitrate [NO_3^-] versus soluble reactive phosphate [SRP] concentration; Dow et al. 2006) or scale of analysis (e.g., land use patterns in the riparian zone versus whole watershed; Dodds and Oakes 2006). Studies examining the effects of LULC on nutrient dynamics of lotic systems most often focus on urban and agricultural land use, as these are considered higher impact land uses (Ekholm et al. 2000, Vanni et al. 2001, Dodds and Whiles 2004, James et al. 2007, Sonoda and Yeakley 2007). Further, ecosystem responses to LULC patterns can vary when moving from low-order streams to higher-order rivers. Land use in the riparian zone of headwater regions can be a strong predictor of downstream water quality even when headwater streams are not flowing; however the strength of the correlation varies with stream order and watershed size (King et al. 2005, Dodds and Oakes 2008). Finally, potential spatial covariation between natural and anthropogenic environmental drivers may complicate investigation of the relationships between LULC and water quality. For example, regional geology can determine the suitability of areas for agricultural use as well as influence stream nutrient concentrations (Allan et al. 1997, Allan 2004, King et al. 2005, Dow et al. 2006).

The major export pathways of nutrients from terrestrial landscapes to riverine systems can be through overland runoff or through hyporheic connection between groundwater and the stream (Dosskey et al. 2010). For N, it is generally thought that dissolved N delivery to rivers and streams is predominantly through groundwater connections, although atmospheric inputs can serve as a substantial

source (Dodds and Oakes 2006, Howarth et al. 2012). At large scales, N flux into aquatic ecosystems is determined by the net anthropogenic N inputs (including fertilizer application, N-fixation by crops, and atmospheric deposition), with fertilizer application typically constituting the largest portion of this load, and on average ~25% of all fertilizer application is exported to rivers (Howarth et al. 2012). In contrast to N, controls of P loading to lotic systems are less well understood, although it is thought that P concentrations are largely a function of surface runoff and storm flow conditions and are only marginally influenced by groundwater exchange (Reddy et al. 1999, Dodds and Oakes 2006, Sonoda and Yeakley 2007, Sharpley et al. 2008, Banner et al. 2009). Additionally, the controls on P loading appear to be context dependent. Dodds and Oakes (2006) found relatively weak LULC control over stream P concentrations, but Banner et al. (2009) found that percentage riparian-scale cropland was a strong predictor of in-stream P concentrations. Landscape influence on in-stream P concentration is highly dependent upon soil type and the amount of particulate loading (Reddy et al. 1999, Calhoun et al. 2002). Larger watersheds have a greater potential for runoff, and thus have the tendency to transport more particulate nutrients and sediments within their downstream reaches (Dodds and Whiles 2004, Bernot and Dodds 2005, Sonoda and Yeakley 2007).

Because of the complexity in both drivers and responses there is a need to move beyond questions that only address land use patterns and the spatial scales at which these patterns most influence riverine ecosystem processes, and to examine the covariation between physiographic environmental gradients (i.e., patterns in climate, geology, and geomorphology) and land use patterns. However, the degree of covariation is rarely addressed (but see Dow et al. 2006) and most researchers focus primarily on the influence of LULC patterns on water quality (Allan 2004). In addition, the majority of studies are conducted on relatively small watersheds that

do not exhibit a substantial range of physiographic environmental gradients (Sliva and Williams 2001, Dodds and Oakes 2006, Dow et al. 2006). In large watersheds, the use of physiographic environmental predictors allows for an examination of how LULC interacts with the naturally occurring environmental gradients within a watershed (Goldstein et al. 2007). Although physiographic environmental variables are sometimes a component in riverscape studies (Sliva and Williams 2001, Dow et al. 2006, Dodds and Oakes 2008), to our knowledge the only study to partition out the effects of LULC and a covarying set of predictors is Dow et al. (2006), who found that the influence of LULC patterns was greater than geologic factors.

In the study presented here, we examined the relationships between physiographic environmental gradients (e.g., location, ecoregion, slope, or stream density), patterns in land use at different spatial scales, and multiple measures of water quality in a large and complex Gulf Slope (USA) river system. Specifically, we examined the combined and individual influences of physiographic environmental gradients and LULC patterns and compared the degree to which these large-scale and relatively static factors influence nutrient concentrations in a large, complex river system (the Brazos River, Texas). We hypothesized that: 1) there would be substantial overlap between physiographic and LULC gradients in the Brazos River watershed, but LULC patterns would be more proximately associated with in-stream water quality and nutrient concentrations and thus have a greater influence on nutrient concentrations within the Brazos River watershed when compared to the effects of physiographic gradients; and 2) that patterns in LULC in both the immediate riparian zone and the individual sub-catchments would most strongly influence in-stream nutrient concentrations, but that the predominant scale and strength of influence would depend upon the specific nutrient parameter and season. Subsequently, we expected land use categories that contain intense human modification (e.g., percent cover of urban development or cultivated agriculture)

to exert the strongest influence on in-stream nutrient concentrations within the watershed (King et al. 2005, Dow et al. 2006).

Methods

Study Region and Catchment Data

The Brazos River spans a distance of 2060 river km from its source near the Texas – New Mexico border to the Gulf of Mexico, and is the 11th longest river in the United States. The Brazos River watershed has a drainage area of ~116,000 km^2 and spans eight ecoregions (Griffith et al. 2004, Zeug and Winemiller 2008, Vogl and Lopes 2009). Our study area covers the lower third of the watershed, covering an area of ~41,000 km^2 (Fig. 1.1). Previous work on the Brazos River watershed has identified a strong longitudinal gradient in long-term mean annual rainfall across the sampling region (Vogl and Lopes 2009, Labay 2010), with sites in the western study area receiving ~79 cm annually and those in the eastern portion of the study area receiving ~114 cm (Table 1.S1). Within the study area, the main-stem of the river is free of impoundments, however, the river upstream and the major tributaries are regulated by dams (Zeug and Winemiller 2008). Our study area encompasses four ecoregions: the Edwards Plateau (EDPL), Texas Blackland Prairie (TBPR), East Central Texas Plains (ECTP), and the Western Gulf Coastal Plains (WGCP). Land use across the entire lower Brazos watershed is predominantly agriculture and grazing (Zeug and Winemiller 2008, Labay 2010); however, the individual sub-watersheds have distinct patterns of land use and environmental gradients (Labay 2010).
For this study, we sampled 33 sites across the lower Brazos watershed, which encompassed a combination of independent small tributaries as well as nested sites along the major tributaries, including the Navasota, Yegua, Little, and Lampasas Rivers. We also sampled four sites along the main-stem of the Brazos River that incorporated physiographic conditions and LULC throughout the entire watershed

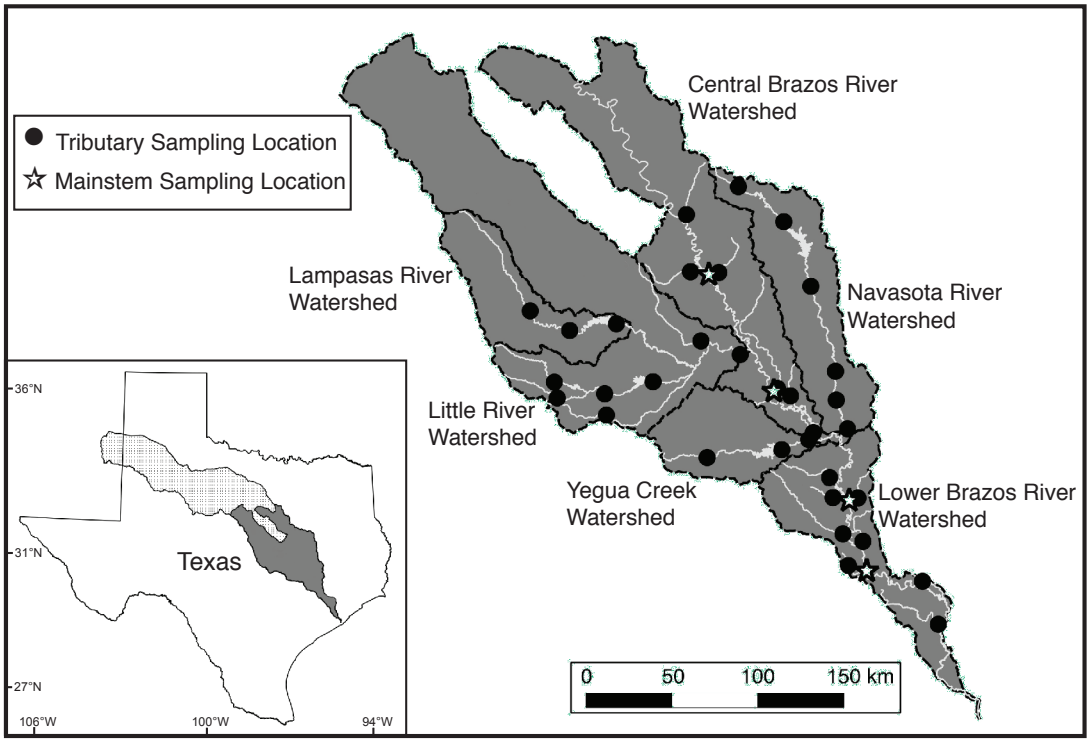

Fig. 1.1. Stream sampling locations and study catchments in the Brazos River watershed in Texas. Inset shows the entire Brazos River watershed. Light stippling indicates the upper Brazos watershed; grey area indicates focus of the present study. Dark lines represent watershed boundaries.

above each site (Fig. 1.1, Table 1.1). Detailed site location information is presented in Supplemental Information Table 1.S1.

To assess LULC patterns at each site, we utilized three spatial scales of analysis that are common in the literature (sensu Allan 2004): 1) Reach-scale, or the "local" scale land use in a 100-m buffer-strip on each side of the channel for a 2 km linear distance upstream from the study site; 2) Riparian-scale, or the intermediate-scale land use in a 100-m buffer-strip on each side of the channel for the entire extent of the watershed upstream of the study site; and 3) Catchment-scale, or the large-scale land use pattern across the entire watershed upstream from the study site. Barren land (e.g., exposed rock or strip-mining areas) was removed from analysis because it generally constituted less than 1% of the total area in the study region (Anderson et al. 1976, Dodds and Oakes 2008). To avoid issues with complete bimodality among LULC predictors, the total LULC was not recalculated

Table 1.1. Watershed, Ecoregion, Physicochemcial, Land Use/Land Cover, and Nutrient data included in the present study

Watersheds	Abbreviation
Central Brazos River	CW
Lampasas River	LM
Little River/San Gabriel River	LR
Lower Brazos River	LB
Mainstem Brazos River	MS
Navasota River	NR
Yegua Creek	YG

Ecoregions	Abbreviation
East Central Texas Plains	ECTP
Edwards Plateau	EDPL
Texas Blackland Prairie	TBPR
Western Gulf Coast Plains	WGCP

Physicochemical Data	Abbreviation	Units
Lattitude	Lat	decimal°
Longitude	Long	decimal°
Catchment Area	C.Area	km^2
Mean Annual Precipitation	MAP	cm
Mean Slope	MSlp	% grade
Max Slope	MxSlp	% grade
Standard Deviation of Slope	sdSlp	% grade
Stream Density	StrDen	km/km^2

Land Use/Land Cover Data	Abbreviation	Units
Cultivated Agriculture	Ag	% cover
Forest	For	% cover
Open Water	O.W.	% cover
Rangeland	Ran	% cover
Urban	Urb	% cover
Wetlands	Wet	% cover

Scaleable Variables	Number following variable
Reach-scale	1
Riparian-scale	2
Catchment-scale	3

Nutrients	Abbreviation	Units
Total Phosphorous	TP	µg/L
Total Nitrogen	TN	µg/L
Soluble Reactive Phosphorous	SRP	µg/L
Particulate Phosphorous	PP	µg/L
Nitrate	NO_3^-	µg/L
Ammonium	NH_4^+	µg/L
Particulate Nitrogen	PN	µg/L
Particulate Carbon	PC	mg/L
Dissolved Organic Carbon	DOC	mg/L
Non-Volitile Suspended Solids	NVSS	mg/L
Suspended Particulate Organic Matte	SPOM	mg/L
Carbon-Nitrogen Ratio (seston)	C:N	molar
Carbon-Phosphorous Ratio (seston)	C:P	molar
Nitrogen-Phosphorous Ratio (seston	N:P	molar
Chlorophyll *a*	Chl *a*	µg/L
Temperature	Temp	°C
Dissolved Oxygen	DO	mg/L
pH	pH	unitless

after removal of barren land.

We assessed scalable physiographic variables at each site at the same three spatial scales used for LULC analyses. At each site, we calculated the landscape maximum slope, mean slope, and standard deviation of slope (a measure of slope variability) using the tools in ArcInfo 9.3 (ESRI, Redlands, CA). Standard deviation of slope has been used as an alternative to mean slope in relatively low gradient watersheds, as is the Brazos (Sliva and Williams 2001). For each site, the non-scalable variables of stream density (stream length per catchment area, km/km^2), catchment area (km^2), and site latitude and longitude (both in decimal degrees) were included as physiographic variables. Season was used as a physiographic variable because seasonal patterns in meteorological variables and riverine discharge are dependent on the physical location within a watershed (Petersen et al. 2012). Finally, season and US EPA Level III ecoregions at the sampling locations were used as categorical physiographic predictor variables. Ecoregions are a practical way to summarize general similarity in large-scale patterns of vegetation type, geology, and other environmental conditions (Griffith et al. 2004). Importantly, the inclusion of site latitude, longitude, sampling season, and to some degree, ecoregion, allowed us to incorporate the effects of spatial- and temporal-structure in the data into the analyses (Borcard et al. 2011). Identification of these trends was one of the goals of this study.

All GIS analyses for the study region were conducted using ArcInfo 9.3. Watershed and catchment delineation was performed using the ArcHydro toolset (Maidment 2002) in ArcInfo. Land use/land cover data were extracted from the 2006 National Land Cover Dataset (NLCD), which is publicly available through the Multi-Resolution Land Characteristics Consortium and USGS National Map Seamless Server (http://seamless.usgs.gov/website/seamless/viewer.htm). The raw data were at a 30m resolution and contained 21 LULC classes. To simplify the dataset

we re-classified the data based on Anderson (1976) Level I, which resulted in seven LULC categories: urban, cultivated agriculture, forest, rangeland (including grasslands), wetland, open water, and barren land. To delineate the watersheds and calculate slope, we used digital elevation models (DEM) from the 2009 National Elevation Dataset at a 1-arc second resolution (~30m), also available on the USGS National Map Seamless Server. Stream network data were from the USGS National Hydrography Dataset (http://viewer.nationalmap.gov/viewer/nhd.html?p=nhd). Stream density was derived from the stream network and DEM derived watershed delineation data. In order to incorporate broad patterns of geology, soil structure, and vegetation into the analysis, sites were assigned to ecoregion according to the US EPA Level III Ecoregions of Texas (http://www.tpwd.state.tx.us/landwater/land/maps/gis/data_downloads/shp/ecomajpy.zip; Omernik 2000, Omernik 2004). Average rainfall for each site was determined with data from the Texas Water Development Board (http://www.twdb.state.tx.us/mapping/gisdata.asp).

Stream Sampling and Laboratory Analyses

Water samples were collected in duplicate from all sites in three field seasons during 2008-2009. Spring sampling occurred March – May 2008; summer sampling occurred June – August 2008; and winter sampling occurred November 2008 – January 2009. Water was collected in acid-washed 2-L brown Nalgene™ bottles, and bottles were rinsed with site water prior to sample collection. Bottles were placed in coolers on ice until processed in the lab within 24 – 48 h of collection. Water temperature (°C), dissolved oxygen (mg/L), specific conductance (μS/cm), and pH were measured at each site with YSI™ sondes (Model 556 or Model 85, Yellow Springs, OH). In the lab, samples were immediately analyzed or divided into subsamples and preserved for future analysis. Water samples were analyzed for total nitrogen (TN), total phosphorous (TP), particulate phosphorous (PP),

particulate C (PC), particulate N (PN), suspended particulate organic matter (SPOM), non-volatile suspended solids (NVSS), dissolved NO_3^-, dissolved ammonium (NH_4^+), dissolved SRP, dissolved organic carbon (DOC), and suspended chlorophyll *a* (chl *a*). Sestonic molar ratios (C:N, C:P, N:P) were calculated from the PC, PN, and PP data.

Total and dissolved nutrient samples were divided into acid-washed polyethylene bottles, acid fixed with concentrated H_2SO_4, and stored frozen until analysis. Total N analysis was done by second derivative spectrophotometry after basic persulfate digestion (Crumpton et al. 1992). Total P analysis was done by the ascorbic acid method after persulfate digestion (Wetzel and Likens 1991). Particulate P samples were determined by filtration onto 47mm pre-combusted Pall A/E filters. For PP analysis, filters were ashed for 2 h at 500°C and subsequently digested with 1N HCl for 1 h at 100°C, and then analyzed by the ascorbic acid method (Wetzel and Likens 1991, Caston et al. 2009). All spectrophotometry was performed on a Varian Cary 50 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA). For determination of PC, PN, and sestonic C:N water was filtered through pre-combusted 25mm Whatman GF/F filters and analyzed on a Thermo Flash EA1112 (Waltham, MA). Suspended particulate organic matter and NVSS were determined by filtration onto a pre-weighed and combusted 47mm Pall A/E filter. After drying for 48 h at 60°C, the sample was reweighed, combusted for 4 hrs at 500°C, and weighed again. Suspended particulate organic matter was calculated as the difference between pre- and post-combustion weights, and NVSS was calculated as the difference between the post-combustion weight and the filter. Dissolved nutrient samples were filtered through pre-combusted Whatman GF/F filters. Nitrate was determined by second derivative spectrophotometry (Crumpton et al. 1992). Soluble reactive phosphorus was determined by the ascorbic acid method (Wetzel and Likens 1991), and NH_4^+ was determined using the phenate method (Wetzel and Likens 1991). Dissolved organic carbon was determined on a Shimadzu

TOC-V_{CSH} (Colombia, MD), using the non-purgeable organic carbon method (APHA 2005) within 48 h of filtration through pre-combusted 47mm Whatman GF/F filters. Chlorophyll *a* was determined by filtering water through a 47mm Whatman GF/F and concentrations were determined by fluorometry after acetone extraction (Wetzel and Likens 1991) on a Turner Designs Trilogy fluorometer (Sunnyvale, CA). For data analysis, the two duplicate samples for each analyte from each site were averaged.

Data Analyses

In order to facilitate the determination of the proportional influences of both physiographic and LULC variables on nutrient dynamics, we initially grouped all predictor variables into two groups, physiographic and LULC (Table S1; sensu Anderson et al. 1976, Dow et al. 2006, Petersen et al. 2012). Here, we define physiographic variables as those that fit under the broad definition of physical geography, which includes data on climatology, geomorphology, and biogeography (Petersen et al. 2012). Land use/land cover data are remote sensed and include both natural and human influenced classes (Anderson et al. 1976). Because of the strength of the correlation between the longitude of a site and the mean annual rainfall ($r^2 = 0.92$, $p < 0.001$), we excluded mean annual rainfall from subsequent principal components analysis (PCA) and redundancy analysis (RDA) and used longitude as a proxy for mean annual rainfall. After grouping variables, we performed PCA on the continuous variables in each of the predictor datasets to evaluate the presence of the physiographic and LULC gradients across the sites in the study area and to assess broad patterns of covariation between predictors within each grouping. Because we had a large number of potential predictor variables to consider for inclusion in subsequent analyses, patterns of covariation in the PCA were used to guide an initial round of data reduction, as it highlighted

correlation between the variables (McCune and Grace 2002). The factor variables of ecoregion and season were not included in the physiographic PCA to avoid problems associated with an excessive variable-to-sample ratio (McCune and Grace 2002). Data were *z*-score standardized and all multivariate analyses were run on a correlation matrix.

When viewing the PCAs as data reduction tools, it was immediately apparent that for nearly all the multi-scale physiographic and LULC predictors (e.g., slope mean, maximum and SD, and the percent cover of different LULC types), the riparian-scale predictors were highly correlated with the catchment-scale predictors and the eigenvectors were of similar length (Figs. 1.2A, 1.3A). The only exceptions to this pattern were with percent forest cover and open water. Percent cover of forest and open water at the riparian- and catchment-scales were correlated, but the strength of these relationships was not as great as with the other predictor variables. Overall, these results indicated that the riparian- and catchment-scales contained similar information and explanatory power. Thus, we elected to run all subsequent models without the riparian-scale predictors.

Redundancy analysis was subsequently used to assess correlations between the remaining physiographic or LULC predictor variables and in-stream nutrient concentrations across the lower Brazos River watershed. Redundancy analysis is a constrained ordination extension of PCA that allows for the selection of predictor and response datasets and variance partitioning (McCune and Grace 2002, Borcard et al. 2011, Legendre and Legendre 2012). Because RDA assumes that predictor-response relationships are linear, RDA is appropriate for environmental predictor – nutrient response datasets (ter Braak and Verdonschot 1995, McCune and Grace 2002, Aufdenkampe et al. 2006). We ran individual (physiographic versus LULC predictors), global (both predictor sets combined), and partial RDAs (both physiographic and LULC predictors, where the analysis is run on one set

of predictors, while controlling for the effect of the second). This last step is the multivariate equivalent of a partial linear regression (Borcard et al. 2011). This allowed us to evaluate through variance partitioning the combined and pure effects of the two predictor sets (King et al. 2005, Peres-Neto et al. 2006, Borcard et al. 2011).

As an initial step in the individual RDAs, we performed a second round of data reduction, where highly correlated predictors in each of the physiographic and LULC categories were identified and removed by back-sequential variance inflation factor (VIF) analysis, where the predictor with the largest VIF was removed and the analysis rerun until all VIF values were < 10 (Dow et al. 2006). High VIF values suggest potential issues with multicollinearity between predictor variables, which can affect the utility of partial regression analyses (Zar 2010, Legendre and Legendre 2012). In the physiographic RDA, the standard deviation of slope at both the reach and catchment-scales were highly multicollinear with the other variables (VIF = 47.7 and 34.0, respectively) and were removed from the analysis. In the LULC RDA, the percentage of cultivated land at the catchment-scale and wetlands at the reach-scale were highly multicollinear with the other variables (VIF = 12886.8 and 136.1, respectively) and were removed from analyses. After removal of these predictors, all VIF were < 7 in both sets of predictors. Permutation tests (minimum n = 200, $\alpha = 0.05$) were run to assess significance of the individual, global, and partial effects models (Borcard et al. 2011, Legendre and Legendre 2012). For all of the RDA models, we present the first two axes corrected by the R^2_{adj}, a more conservative measure of explanatory power than the commonly reported "proportion of inertia explained" (Peres-Neto et al. 2006, Borcard et al. 2011).

Finally, we used linear regression on the annual average data to summarize the univariate relationships between nutrients, physiographic, and LULC data. Data for each constituent nutrient was averaged for each site across sampling seasons.

In order to be as comparable as possible to the multivariate analyses and to avoid predictors with high VIFs, we used the predictor datasets used in the final separate physiographic and LULC RDAs. The best performing model for each nutrient was selected using the minimum Akaike's information criterion corrected for small sample size (AICc; Burnham and Anderson 2004). A forward selection procedure was used and the categorical variable of ecoregion was assessed using the whole effect rule where it is only added to the model if all levels reduce the AICc. This resulted in two predictor models for each nutrient. In the RDA and linear regression analyses, response variables (nutrients) were $\log_{10}$ transformed when needed to meet the assumption of normality in response distributions. All univariate statistics were performed using JMP 9.0 (SAS, Inc., Cary, North Carolina). Multivariate ordination (PCA and RDA) and variance partitioning were performed using the 'vegan' package in the R statistical environment (Oksanen et al. 2012, R Core Team 2013).

Results

Physiographic Gradients and Regional LULC Patterns

The PCA of physiographic variables accounted for 73.7% of the variation among sites in the first two axes (Fig. 1.2). Principal component 1 (PC1) explained 58.8% of the variation among sites, with all measures of slope (mean, maximum, and standard deviation) at all scales (reach-, riparian-, and catchment-) having qualitatively similar influence along this axis. Loadings with a narrow range of 1.3-1.6 suggested that the three measures of slope were approximately equivalent in terms of explanatory power. In general, PC1 represented a gradient of sites with greater stream density and longitude (with negative loadings on PC1) to sites with greater mean, maximum, and variability of slope (with positive loadings on PC1). Study sites effectively oriented in an east-to-west gradient in the Brazos watershed

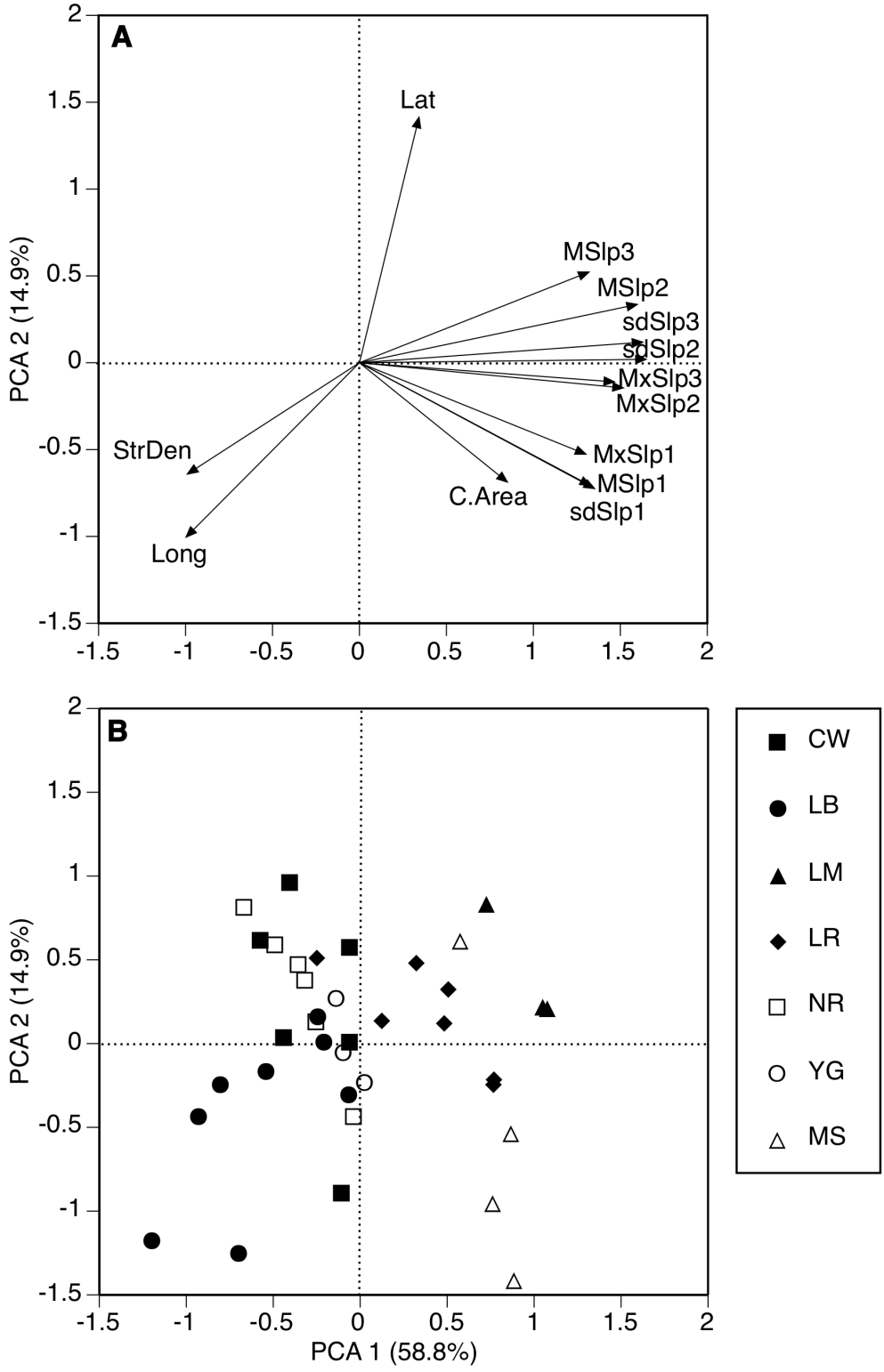

Fig. 1.2. Principal component analysis of the physiographic variables used in the present study. See text for selection procedures. A. – Multivariate relationships between the physiographic variables. Abbreviations are consistent with Table 1.1. Spatial arrangement of the study catchments is shown on Fig. 1.1.

on PC1. Principal component 2 (PC2) also represented a geographic gradient of more southern and western sites within the watershed (i.e., lower latitude and greater longitude) combined with a geomorphic gradient of higher stream density and larger catchment areas in the southern portions of the watershed (represented by negative loadings along PC2) to more northern sites (i.e., greater latitude; represented by positive loadings on PC2). Thus, the PCA for physiographic data essentially detailed the geographic positioning of sites within the Brazos watershed, with the combined gradients representing the watershed-scale variation of southeastern-positioned, higher rainfall, lower landscape slope systems to northwestern-positioned, steeper slope sites with lower annual rainfall.

Similarly, the LULC PCA also described a large-scale geographic gradient in patterns of LULC throughout the Brazos River watershed (Fig. 1.3). Collectively, the first two axes explained 57.8% of the variation among sites in LULC. Principal component 1 accounted for 37.7% of the variation among sites and described a gradient of sites characterized by catchment- and riparian-scale cultivated land or a greater percentage of wetland area (represented by negative loadings on PC1) to a greater proportion of catchment- and riparian-scale rangeland and forest cover (represented by positive loadings on PC1). In general, this axis described watershed-scale patterns in LULC spanning in a southeastern to northwestern direction, as well as a land use intensity gradient, with sites in the lower portion of the watershed characterized by cultivated land and the presence of wetlands and sites in the upper portion of the watershed characterized by forested area, rangelands, and few wetlands. Principal component 2 represented a gradient of sites with higher percentages of catchment-scale open water and reach-scale wetlands area (represented by negative loadings on PC2) to sites with higher percent catchment- and riparian-scale urban and reach-scale cultivated land use (represented by positive loadings on PC2). This axis also portrayed the variation in site-level LULC

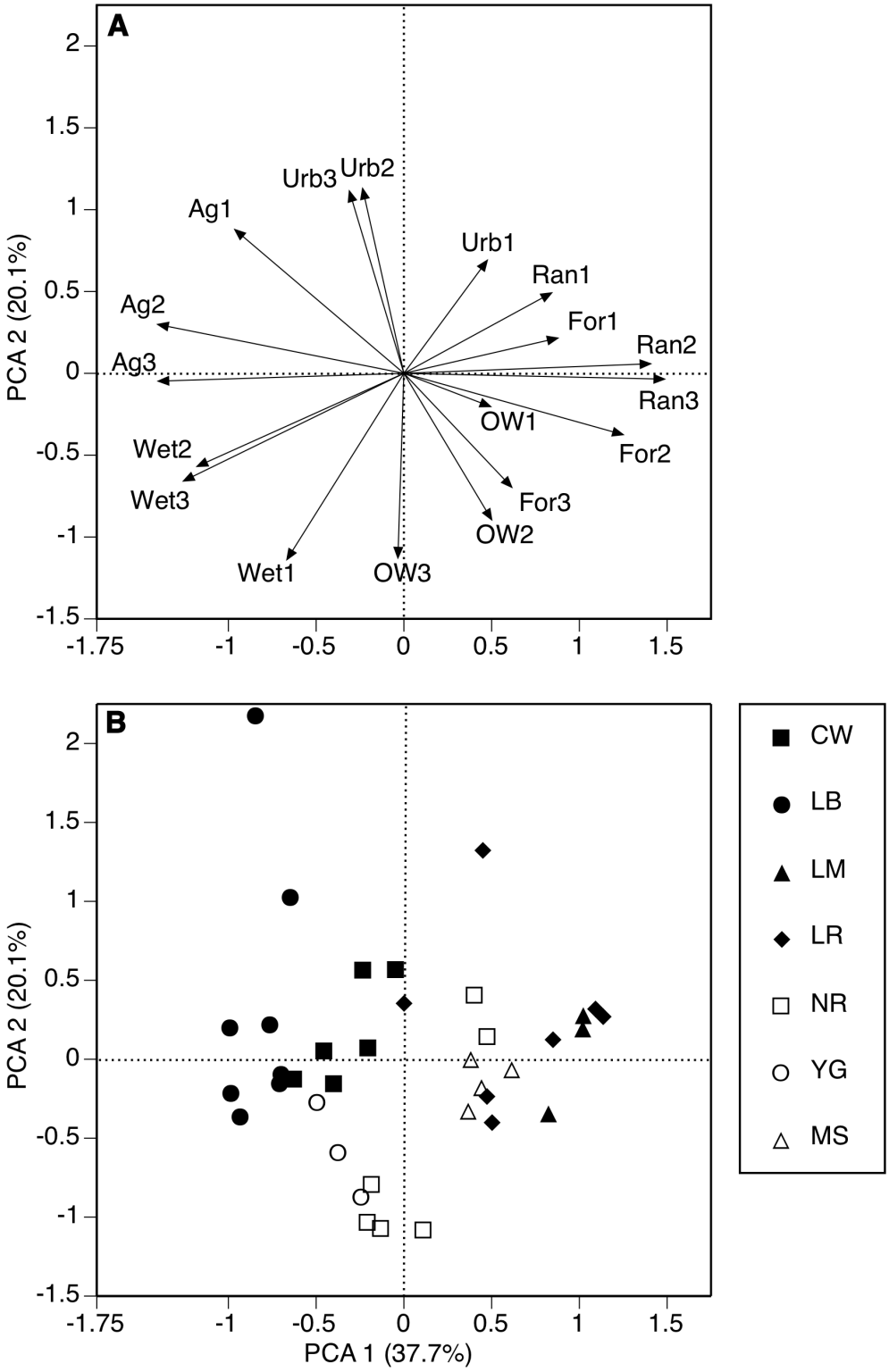

Fig. 1.3. Principal component analysis of the LULC variables used in the present study. See text for selection procedures. A. – Multivariate relationships between LULC variables. Abbreviations are consistent with Table 1.1. Spatial arrangement of the study catchments is shown on Fig. 1.1.

within the individual sub-watersheds of the Brazos River. For example, sites in the lower Brazos tributaries (LB sites in Fig. 1.3B) typically have low rangeland and forest cover and higher levels of cultivation (i.e., consistent positions along PC1), but the individual sites within this section of the Brazos varied greatly in their urban versus wetland coverage (i.e., variable positions along PC2). In fact, for all of the sub-watersheds, variability along PC2 was greater than the variability along PC1, highlighting the difference between regional and local LULC gradients.

Nutrient Responses to Physiographic and LULC Gradients

The first two physiographic RDA axes accounted for 27.1% of the variation in nutrient concentrations and $R^2_{adj} = 0.39$ ($p < 0.005$) for the entire model (Fig. 1.4A). The first RDA axis explained 17.8% of the variation in the nutrient data and largely represented a southeast – northwest gradient in the Brazos watershed. Southeastern sites in the WGCP ecoregion were characterized by higher concentrations of total and dissolved nutrients (TP, TN, SRP, NO_3^-, NH_4^+), which were positively correlated with stream density and site longitude. In contrast, the more northwestern sites in the EDPL ecoregion were characterized by higher reach-scale maximum slope and reach- and catchment-scale mean slope, which were negatively correlated with total and dissolved nutrients and positively correlated with higher seston C:P and N:P (indicating lower P content of seston). The second axis of the physiographic RDA (RDA2) was strongly influenced by catchment-scale maximum slope and catchment area. Particulate matter and algal biomass (e.g., PC, PN, SPOM, NVSS, and suspended chl *a*) and higher seston C:N were positively correlated with catchment area and samples collected in the spring. Finally, the physiographic RDA also indicated that samples taken in the summer and in the ECTP and TBPR ecoregions showed the least deviation from the mean values across sites.

The first two LULC RDA axes accounted for 20.6% of the variation in the

data and R^2_{adj} = 0.25 (p < 0.005) for the entire model (Fig. 1.4B). The first RDA axis explained 13.4% of the variation in the nutrient data and represented a gradient of increased forest and rangeland LULC (at both the reach- and catchment-scales) to sites with higher percent cover of catchment-scale urban LULC and reach-scale cultivated area. Forest and rangeland were correlated with low total and dissolved nutrients and higher seston C:P and N:P while urban and cultivated LULC were correlated with greater total and dissolved nutrients. Much like patterns observed in the RDA for physiographic predictors, the first LULC RDA axis represented a general northwestern – southeastern spatial gradient across the Brazos River watershed. The second axis (RDA2) of the LULC RDA portrayed a gradient of sites with greater percentage of open water (especially at the reach-scale) to sites with higher coverage of reach-scale urban LULC. Along this axis, greater open water coverage was correlated with higher particulate concentrations (PC, PN, SPOM, and NVSS) and suspended chl *a*, whereas urban coverage at the catchment-scale was positively correlated with greater dissolved nutrient concentrations (especially NH_4^+ and SRP) and lower concentrations of suspended materials. Dissolved oxygen, pH, DOC, and water temperature all had relatively weak responses to LULC parameters.

When physiographic and LULC predictor sets were combined into a global RDA model (Fig 1.4C), the first two axes accounted for 32.9% of the variation in the data and R^2_{adj} = 0.51 (p < 0.005) for the complete model. The first RDA axis explained 20.2% of the variation in the nutrient data. The global RDA showed a high level of correlation between physiographic and LULC predictors across the Brazos River watershed (Fig. 1.4C). Sites with higher stream density were associated with higher levels of reach-scale cultivated LULC and catchment-scale urban coverage; these predictors were positively correlated with nutrient concentrations (both total and dissolved fractions) and these sites more commonly occurred in the WGCP ecoregion. Reach-scale percent urban land use was correlated with elevated DO,

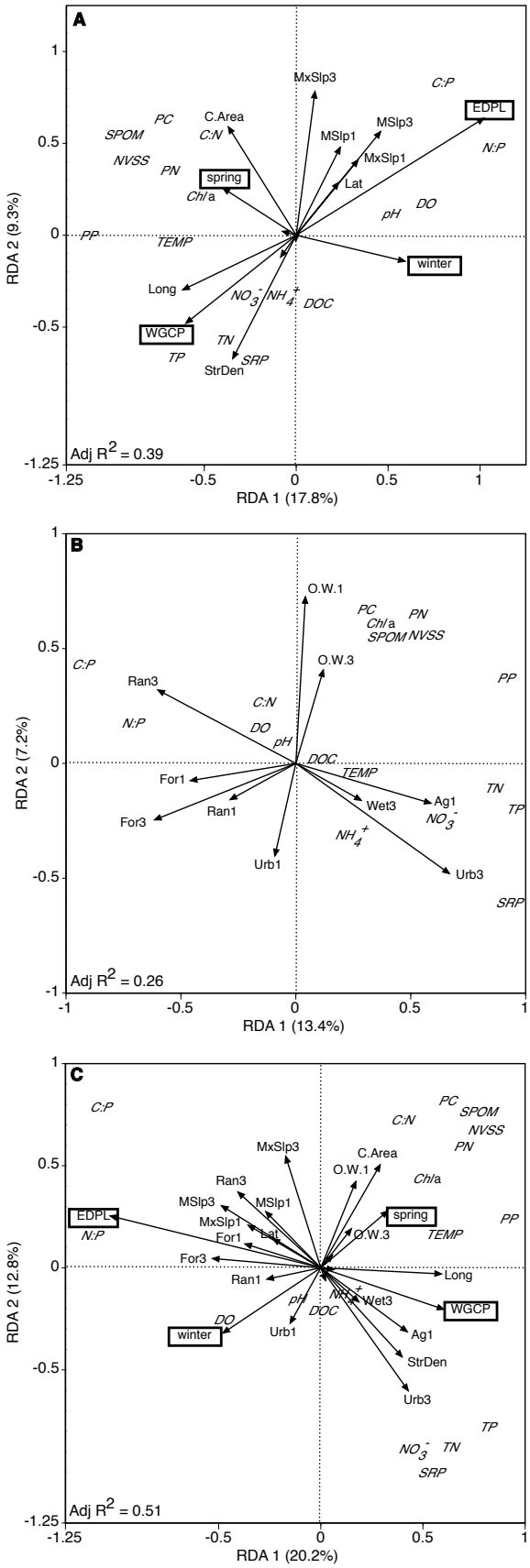

Fig. 1.4. Redundancy Analysis plots of the relationships between predictor groups and nutrient concentrations in the Brazos River. Abbreviations are consistent with Table 1.1. Nutrient response variables are indicated by italics. Scaling may be slightly adjusted for readability. See text for selection procedures. A – Nutrient relationships to physiographic variables. ECTP, TBPR and summer sampling season are not indicated because they are located very near the origin. B – Nutrient relationships to LULC predictors. C – Combined "global" analysis including both groups of predictors. ECTP, TBPR and summer sampling season are not indicated because they are located very near the origin.

pH, and DOC, and these variables were also elevated in the winter sampling season. More forested sub-watersheds (at both scales) and catchment-scale rangeland are correlated with steeper and more variable slopes (at both scales), and these sites predominantly occurred in the EDPL ecoregion and have higher seston C:P and N:P. Catchment area was positively correlated with open water at both scales, and suspended particulate materials, suspended chl *a*, and seston C:N were higher in these larger watersheds, notably during the spring sampling season.

Partial Effects and Variance Partitioning of Physiographic and LULC Data

The RDA used to assess the pure effects of physiographic variables, after controlling for the influence of LULC parameters, explained 16.4% of the variation in the nutrient and water quality data within the first two axes, and the $R^2_{adj} = 0.25$ ($p < 0.005$; Fig. 1.5A). When the effect of LULC is removed, total nutrients, SRP and NO_3^- were positively correlated with latitude and generally higher in the TBPR ecoregion, but were negatively correlated with stream density. In contrast, NH_4^+ and C:P were positively correlated with stream density and were generally higher in the summer season and at sites in the in both the EDPL and WGCP ecoregions. In this partial RDA, SPOM was still positively correlated catchment area and was additionally elevated in the spring season. Seston N:P and DOC were positively correlated with winter season sampling, but were negatively correlated with catchment area and the spring season. This analysis also indicated that the maximum and mean slope of sites were relatively weak predictors of nutrient concentrations, as was longitudinal position in the watershed.

The RDA used to assess the pure effects of LULC predictors (after controlling for physiographic parameters) explained 9% of the variation in the nutrient and water quality data within the first two axes, with the model's $R^2_{adj} = 0.12$ ($p < 0.005$; Fig. 1.5B). Concentrations of TN and NO_3^- responded strongly and positively to

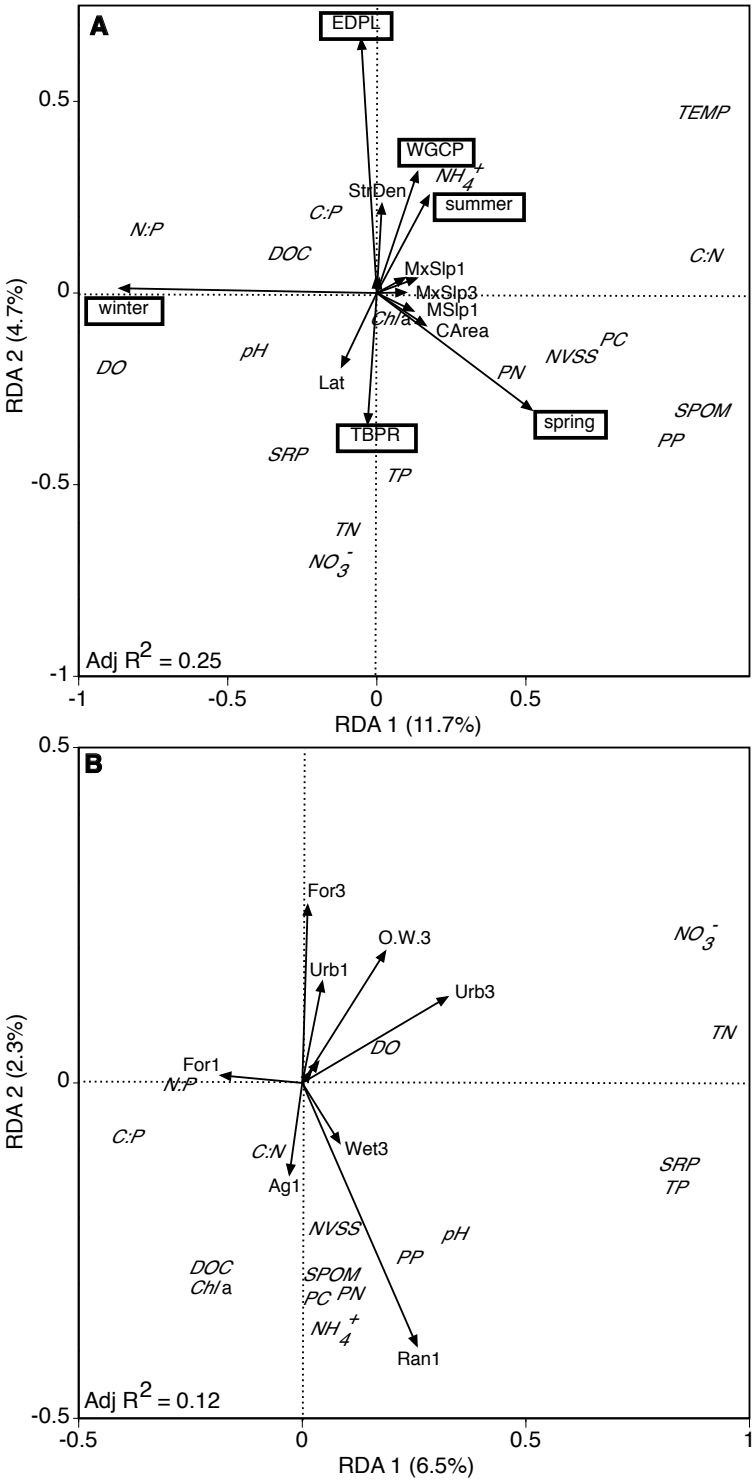

Fig. 1.5. Partial Redundancy Analysis plots of the relationships between predictor groups and nutrient concentrations in the Brazos River. All abbreviations are consistent with Table 1.1. Scaling may be slightly adjusted for readability. A – Nutrient relationships to physiographic predictors after accounting for the influence of LULC predictors. ECTP ecoregion, Long, and MSlp3 are not indicated because they are located very near the origin. B – Nutrient relationships to LULC predictors after accounting for the influence of physiographic predictors. Wat1, Ran3, and TEMP are not indicated because they are located very near the origin.

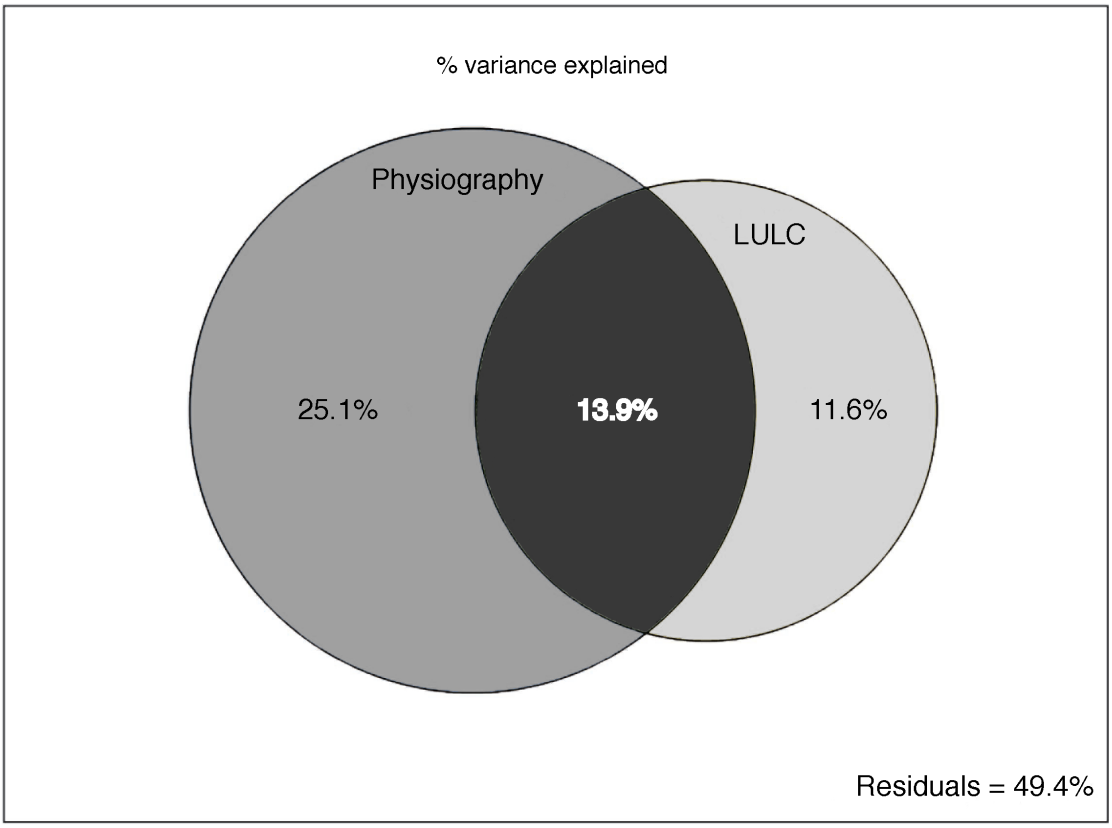

Fig. 1.6. Results of the variance partitioning analysis showing the proportion of independent and combined influence of each predictor group as well as the unexplained variance of nutrient concentrations (Residuals).

catchment-scale urban land use. Total P and SRP were also positively influenced by catchment-scale urban land use, but were additionally affected by percent cover of reach-scale rangeland. Suspended particulate matter and NH_4^+ concentrations were correlated with reach-scale rangeland, whereas, suspended chl *a*, DOC, and seston C:N were most strongly correlated with reach-scale cultivated land. Seston C:P and N:P were positively associated with reach-scale forest cover. In this analysis, reach-scale open water and catchment-scale rangeland had weak influence on nutrient concentrations and water quality conditions.

Although both physiographic and LULC RDAs explain significant amounts of the variation in nutrient and water quality data, variance partitioning indicates that physiographic parameters accounted for 25.1% of the variation, approximately

Table 1.2. Results of multiple regression analyses testing the ability of physiographic and LULC variables to predict in-stream nutrient concentrations. The models with the lowest AICc score are listed. CR = coefficient of regression for the selected predictors. Coefficients for Ecoregion are not given as each ecoregion can have individual values, and it was included as a whole effect only. Bold indicates p <0.05. Abbreviations are consistent with Table 1.1.

Physiographic Models

Nutrient Response	Best Model	AICc	R^2_{adj}	*CR*	*p*
TP	Long (+), StrDen (+)	122.9	0.32	0.81, 2.00	**<0.001**
TN	MSlp 3 (-)	108.3	0.10	-0.63	**0.033**
SRP	StrDen (+), MxSlp 3 (-)	137.0	0.25	1.79, -0.04	**0.003**
PP	Lat (+), Long (+), C.Area (+), StrDen (+)	69.8	0.60	0.62, 0.66, 0.00002, 1.68	**<0.001**
NO_3^-	StrDen (+)	138.5	0.05	1.62	0.110
NH_4^+	StrDen (+)	37.9	0.19	0.78	**0.004**
PN	Lat (+), Long (+), C.Area (+), MxSlp 1 (-)	61.0	0.50	0.55, 0.62, 0.00001, -0.001	**<0.001**
PC	Lat (+), Long (+), C.Area (+)	73.2	0.49	0.50, 0.41, 0.00002	**<0.001**
DOC	MxSlp 1 (-)	46.4	0.23	-0.033	**0.002**
NVSS	Lat (+), Long (+), C.Area (+)	126.9	0.48	1.38, 1.87, 0.00002	**<0.001**
SPOM	Lat (+), Long (+), C.Area (+)	78.4	0.49	0.64, 0.71, 0.00002	**<0.001**
C:N	Lat (+), C.Area(+), MSlp 1 (+), MSlp 3 (+), Ecoregion	1.8	0.56	0.15, 0.000004, 0.04, 0.20	**<0.001**
C:P	Long (-), StrDen (-), MSlp 1 (+), MSlp 3 (+)	31.0	0.69	-0.19, -1.04, 0.09, 0.21	**<0.001**
N:P	StrDen (-), MxSlp 1 (+), MSlp 3 (+)	35.4	0.60	-0.95, 0.02, 0.32	**<0.001**
Chl *a*	Lat (+), C.Area (+), MxSlp 1 (-), Ecoregion	100.9	0.46	0.76, 0.00002, -0.002	**<0.001**
Temp	Lat (-), Long (+)	145.4	0.58	-1.65, 1.85	**<0.001**
DO	Long (-), C.Area (+)	159.3	0.26	-1.83, 0.00002	**0.002**
pH	Long (-)	19.5	0.16	-0.20	**0.014**

LULC models

Nutrient Response	Best Model	*AICc*	R^2_{adj}	*CR*	*p*
TP	Urb 1 (-), Urb 3 (+), For 3 (-), Wets 3 (+)	102.6	0.64	-0.08, 0.10, -0.048, 0.12	**<0.001**
TN	Urb 1 (-), Urb 3 (+)	94.7	0.40	-0.05, 0.08	**<0.001**
SRP	Urb 1 (-), Urb 3 (+)	126.4	0.44	-0.13, 0.14	**<0.001**
PP	Ag 1 (+), O.W. 1 (+), For 3 (-), O.W. 3 (+)	79.9	0.48	0.02, 0.02, -0.03, 0.50	**<0.001**
NO_3^-	Urb 3 (+)	124.4	0.35	0.09	**<0.001**
NH_4^+	Urb 3 (+), O.W. 3 (-)	40.7	0.16	0.01, -0.18	**0.019**
PN	Ag 1 (+), O.W. 1 (+), For 3 (-), O.W. 3 (+)	65.2	0.44	0.01, 0.02, -0.02, 0.51	**<0.001**
PC	O.W. 1 (+), For 3 (-), O.W. 3 (+)	79.8	0.44	0.03, -0.03, 0.34	**<0.001**
DOC	Ran 1 (+), Urb 3 (-), Ran 3 (-)	50.9	0.20	0.01, -0.01, -0.01	**0.016**
NVSS	Urb 1 (-), For 3 (-), O.W. 3 (+)	123.7	0.52	-0.08, -0.10, 1.25	**<0.001**
SPOM	Urb 1 (-), O.W. 1 (+), For 3 (-), O.W. 3 (+)	83.5	0.45	-0.03, 0.02, -0.04, 0.47	**<0.001**
C:N	Ran 3 (+), For 3 (-)	1.1	0.48	0.01, -0.01	**<0.001**
C:P	Urb 1 (+), For 1 (-), Urb 3 (-), Ran 3 (+)	22.3	0.76	0.03, -0.006, -0.04, 0.02	**<0.001**
N:P	Urb 1 (+), Urb 3 (-), Ran 3 (+), For 3 (+)	28.0	0.69	0.03, -0.03, 0.01, 0.02	**<0.001**
Chl *a*	O.W. 1 (+), For 3 (-), O.W. 3 (+)	100.8	0.38	0.02, -0.05, 0.62	**<0.001**
Temp	O.W. 1 (+), Ran 3 (-)	159.0	0.40	0.05, -0.08	**<0.001**
DO	Ran 3 (+)	158.6	0.25	0.05	**0.001**
pH	Ran 1 (+), O.W. 1(+)	14.1	0.28	0.01, 0.007	**0.001**

twice that of the independent explanatory power than LULC variables (Fig. 1.6). There was substantial overlap in the two datasets (13.9%), which illustrates the extent to which many of these predictors are collinear. Much of this collinearity can be attributed to three apparent groupings of predictors (Fig. 1.4C): 1) Reach-scale mean and maximum slope and catchment-scale mean slope are highly correlated

with both scales of forest and catchment-scale range LULC; 2) Catchment area and open water (at both scales) are highly correlated; and 3) Stream density is highly correlated with reach-scale cultivated and catchment-scale urban LULC. Despite this, both physiographic and LULC parameters provide substantial independent explanatory power and combine to explain over half of the variation in nutrient conditions throughout the Brazos River watershed.

The linear regression models selected by the stepwise process identified largely similar important sets of predictors in each group as the multivariate analyses (Table 1.2). This is especially true for the predictors that had strong ordination on the first axes of the RDAs. Significant models were found for every nutrient except the NO_3^- - physiographic combination. Model selection did explicitly identify some of the negative correlations between predictors and nutrient responses that might be visually missed in the RDA figures, however because of the similarity of results and ease of graphical interpretation of the RDAs, we concentrate our discussion on the results of the multivariate analyses.

Discussion

Physiographic Gradients and Regional LULC Patterns in the Brazos River Watershed

In this study, we observed large-scale spatial variation in both physiographic and LULC characteristics across the Brazos River watershed. Additionally, patterns in physiographic and LULC gradients covaried spatially, with both sets of data approximating the northwest-to-southeast spatial arrangement of sites in the watershed. If an investigator were to solely concentrate on LULC patterns in the lower Brazos, as is often the case in in smaller watershed studies (Sliva and Williams 2001, Dodds and Oakes 2006, 2008), it would appear that the primary gradient within the watershed was one based on land use intensity (e.g., cultivated agriculture versus forest and rangeland cover; Figs. 1.3B, 1.4B). However, the

addition of physiographic data to our analysis of this large complex watershed revealed LULC patterns that appear to be strongly influenced by existing natural gradients within the lower Brazos watershed. The northwestern portions of the watershed, primarily located in the Lampasas and upper Little River sub-watersheds exhibit greater aridity, more variable topography, and the regional geology and soils typical of the EDPL ecoregion, which is characterized by shallow limestone bedrock with little topsoil development (Barnes 1992, Griffith et al. 2004, NRCS 2008). Consequently, much of the LULC patterns in EDPL ecoregion of the Brazos watershed are typical of more low-intensity activities, in that they exhibit higher percentages of forest and rangeland cover. In contrast, the more gentle topography and higher annual precipitation of the southeastern portion of the watershed in the WGCP ecoregion was correlated with higher stream density and deep, often clayey soils (Barnes 1992, Griffith et al. 2004, NRCS 2008). Higher annual precipitation and greater connection with the floodplain was subsequently associated with higher percentages of agricultural and urban LULC at both the riparian- and catchment-scales (Figs. 1.2, 1.3).

The second gradient we observed across the lower Brazos watershed was essentially driven by stream order (Fig. 1.4C), as it positively correlated with both catchment area and the percent cover of open water (Kalff 2001). That catchment area and percent cover of open water would be correlated is not surprising but results from the present study highlight the difficulty in separating out the influence of either as an independent driver of river condition. Both physiographic and LULC predictors describe a substantial portion of the variation between sites, however measures of physiographic condition explain substantially more (73.7% vs. 58.8% in the first two PCA axes) than LULC. Further, the large spatial extent of this study allowed us to identify natural physiographic gradients that explained a substantial portion of the nutrient concentration patterns, and contrary to our first hypothesis,

more than the LULC gradients explained. Results from our study indicate that physiographic patterns and context can strongly influence the spatial arrangement of LULC and highlights the importance of considering both groups of data in large riverscape studies (Allan 2004, King et al. 2005).

Nutrient Responses to Physiographic and LULC Gradients

In this study, large watershed-scale patterns in physiographic gradients exhibited a strong influence on the spatial patterns of in-stream nutrient concentrations in the Brazos River watershed. As we expected, the responses were variable as to the predictor with which they were most correlated. Across the study area, in-stream nutrients (e.g., N and P) tended to be dominated by the dissolved fractions (the sum of NH_4^+ and NO_3^- for N and SRP for P) accounting for 71% and 94% of the total N and P pools, respectively, for the study period (Table 1.S2). However, higher concentrations of both total and dissolved nutrients were positively correlated with longitudinal position in the Brazos watershed (and thus mean annual rainfall) and stream density (Fig. 1.4B); spatially, these sites were largely situated in the WGCP ecoregion. In-stream TN, NO_3^-, and NH_4^+ concentrations were positively correlated with stream density, and negatively correlated with slope; these conditions increase the land-water contact and decrease flow velocity, and thus increase the opportunity for groundwater interaction in areas which have presumably high N inputs from agricultural practices (Dodds and Oakes 2006, Dosskey et al. 2010, Filoso and Palmer 2011, Howarth et al. 2012).

We found that in-stream TP, SRP, and to a lesser degree, PP, were higher in the eastern portions of the Brazos watershed where mean annual rainfall is higher. In the more eastern WGCP ecoregion, deep, clay soils dominate (Barnes 1992, Omernik 2000) and erosional processes deliver relatively high P concentration sediments to the river (Sharpley and Smith 1983, Kalff 2001, Calhoun et al. 2002, Haggard et

al. 2003). Additionally, groundwater in the EDPL ecoregion is often low in P while exhibiting elevated carbonate concentrations (Groeger and Gustafson 1994, Groeger et al. 1997). These conditions are typical of waters in areas of high limestone weathering, which can further reduce P concentrations in streams during baseflow conditions through the co-precipitation of P with calcite into travertine (Reddy et al. 1999, Wetzel 2001). Additionally, the seston C:P and N:P ratios were elevated in the more arid EDPL ecoregion, indicating low P availability in streams .

Across the study region, concentrations of particulate matter (particulate C, N, P, SPOM, NVSS, and, suspended chl *a*) were strongly correlated with catchment area and the spring sampling season, and all of these responses generally increased along the west-to-east rainfall gradient in the watershed. This pattern is consistent with conceptual models of riverine function and empirically derived data of riverine systems (Vannote et al. 1980, Wetzel 2001). Higher discharge in the downstream and eastern portions of the Brazos watershed, especially during the relatively wetter spring season, would tend to enhance this this pattern (Sharpley et al. 2008, Banner et al. 2009). The springtime average flow at the USGS gauging station nearest the most downstream sampling location was ~4× the summer sampling and ~7× than the winter sampling. Additionally, C:N of suspended particulate material was highly correlated with catchment area, a pattern which is consistent with increasing inputs of allochthonous refractory C material with low N content from the watershed (Wildhaber et al. 2012). Although particulate material and nutrients were likely predominantly allochthonous in origin, the large size of the lower reaches in the Brazos River and its tributaries precludes full canopy cover, and there were greater concentrations of suspended chl *a*, indicating increased in-stream autochthonous production in these reaches (Mulholland et al. 2001, Ensign et al. 2012).

Although we were not able to directly assess differences between the scales of cultivated agricultural land use due to issues of multicollinearity, the high degree

of correlation between the scales suggests that the impact of agricultural land use is similar whether looking at the reach- or catchment-scale. However, with urban land use, there does appear to be a substantial difference in effect between the two different scales, with local-scale urban land use negatively correlated with particulate responses, and catchment-scale urban land use positively correlated with total and dissolved nutrients (Figs. 1.4B, 1.5B). Below we discuss the potential reasons for this in relation to P compounds, but it likely applies to broader groups of dissolved versus particulate nutrients (Dodds and Whiles 2004). These LULC types are often the focus of studies because they are considered relatively high-intensity, resulting in large impacts on hydrology and riverine nutrient concentrations (Ekholm et al. 2000, Vanni et al. 2001, Sonoda and Yeakley 2007, Banner et al. 2009).

We examined the influence of a variety of LULC types, including agricultural and urban LULC, and found that the percent cover of reach-scale cultivated land was significantly associated with increased in-stream TN, NO_3^-, and TP concentrations. This result is consistent with a number of other studies (Dodds and Oakes 2006, Arango and Tank 2008, Dodds and Oakes 2008, Banner et al. 2009). For example, Dodds and Oakes (2006, 2008) found that near-stream agricultural LULC led to higher in-stream TN and NO_3^- concentrations, and that the increase in NO_3^-could be as much as 10× higher in areas with actively fertilized agriculture. Because NO_3^- is typically highly mobile in soils, NO_3^- applied as fertilizer enters aquatic systems in a dissolved and labile form (Haggard et al. 2003).

It is often assumed that urban land use should increase particulate inputs to streams, however Dodds and Whiles (2004) suggested that 1) urban land use may reduce sediment loading to streams by reducing the amount of exposed erodible soil, and 2) that the effects of urban land use may attenuate quickly downstream. This would potentially explain the predominantly negative correlation between reach-scale urban LULC and PP observed (as well as the other particulate variables;

Fig. 1.4B). Additionally, reach- versus catchment-scale effects may not be mutually exclusive. We found a close correlation between TP and reach-scale cultivated LULC as well as a close correlation of SRP and catchment-level urban LULC. Other studies have found a positive correlation between urban LULC and SRP in small to medium sized watersheds (Brett et al. 2005, Sonoda and Yeakley 2007). Reach-scale urban LULC may have a quickly attenuated effect on PP that is that is largely independent of, or overridden by, the regional affect catchment-scale urban LULC has on SRP, the dominant fraction of P in these systems.

In this study, NH_4^+ was correlated with both reach- and catchment-scale urban LULC and ordinated approximately midway between these two scales (Fig. 1.4B). These findings are similar to Sliva and Williams (2001) who found a correlation with both reach- and catchment-scale percentage urban land use during the spring and summer. Similarly, Dow et al. (2006) found that the most common variable selected to predict in-stream NH_4^+ concentrations was the percent of commercial land coverage (a category of urban land use). The correlation between NH_4^+ and urban LULC is often attributed to wastewater treatment plants, leaky sewer and septic systems, and runoff derived from automobile traffic (Paul and Meyer 2001, Hope et al. 2004, Bernhardt et al. 2008).

Suspended particulate matter was correlated with percent cover of open water (at both the reach- and catchment-scales), which is likely a consequence of stream size or order (Wetzel 2001). Sites with greater open water area were typically the larger-order main-stem Brazos River sites. This is further supported by the correlation with suspended chl *a*, as primary production is often correlated with open canopy (Grimm et al. 2005). This is consistent with larger watersheds having a greater potential for runoff, and transporting more fine particles and sediment in their downstream reaches (Ekholm et al. 2000, Wetzel 2001, Dodds and Whiles 2004, Bernot and Dodds 2005).

We observed that seston C:P and N:P were positively correlated with catchment-scale rangeland. Grimm et al. (2005) found that N:P was positively correlated with urban land use, contrary to N:P patterns in our study. This may be explained by grasses (the dominant plant in rangeland) retaining more P in the watershed than forest cover, which would elevate both C:P and N:P (Osborne and Kovacic 1993, Sliva and Williams 2001). Seston C:P could also be responding negatively to reach-scale cultivated land (James et al. 2007). The correlation of C:P and N:P with catchment-scale rangeland suggests that rangelands may be more efficient at retaining P than forest cover. Allochthonous inputs are likely to be relatively elevated in C, whereas N delivery is likely to be provided through groundwater and nutrient recycling pathways (Wetzel 2001, Arango and Tank 2008). Additionally the responses of PC and PN are smaller than PP along RDA1 (Fig. 1.4B), suggesting that in the Brazos River the more variable component in the ratios is PP, and that the C:P and N:P ratios are more influenced by changes in PP concentration than by variability in either PC or PN (Osborne and Kovacic 1993, Haggard et al. 2003).

Partitioning the Effects of Physiographic and Land Use Gradients

Very few studies have attempted to parse out the individual and combined effects of physiographic or geologic parameters and LULC predictors on riverine nutrient conditions (but see Dow et al. 2006) even though it is well know that the land use and physiographic conditions in a watershed are frequently coupled (Allan 2004, King et al. 2005). The present study highlights the high degree of covariation among physiographic and LULC characteristics in the Brazos watershed (Fig. 1.4C). The primary covariation we observed was related to the regional spatial variation in physiography and LULC patterns overlaid across two of the more prominent ecoregions in the watershed (the EDPL and the WGCP). The primary physiographic

gradient (stream density and eastern locations to steeper slopes and western locations) was aligned with a modification intensity gradient of LULC (Fig. 1.4). Sites in the WGCP ecoregion tended to have lower slopes, higher stream density, catchments with greater levels of urban land use and a greater percent cover of reach-scale cultivated land. In contrast, sites in the EDPL ecoregion had steeper slopes and more forest and rangeland cover. Secondarily, there was a watershed-size or stream-order gradient (Fig. 1.4). This gradient is additionally overlaid across a seasonal gradient, with the effect of larger catchments/higher stream-order sites with higher particulate loading being exacerbated in the wetter spring sampling. These findings highlight the issues raised by Alan (2004) and King et al. (2005), in that LULC is not independent of potentially underlying factors such as climate or geology, and indicate that further investigation into the degree of covariation between physiographic and LULC landscape features is needed.

The use of variance partitioning allowed us to assess the effects of physiographic predictors after controlling for LULC predictors and vice versa (Peres-Neto et al. 2006, Borcard et al. 2011). When the influence of LULC factors was controlled, different patterns of physiographic influences on the nutrient conditions became apparent. In the absence of LULC influences, the effect of season (especially spring and winter) became one of the primary influencing gradients on in-stream nutrients. Catchment area and spring sampling were correlated with higher suspended particulate materials, but the reach-scale mean slope was now associated with these higher in-stream particulate nutrients. This finding was more consistent with Sliva and Williams' (2001) correlation between total suspended solids and slope variability. When the effects of LULC were controlled for, the effects of ecoregion became secondary, along with several other physiographic factors, namely the latitudinal position of the site in the watershed and stream density (Fig. 1.5A). The TBPR ecoregion became associated with elevated TN, NO_3^-, TP, and SRP,

whereas in the unconditioned analysis there was little observable TBPR effect. The WGCP ecoregion was still associated with elevated NH_4^+ concentrations, as is the summer season. Dodds and Oakes (2008) found that the Western Corn Belt Plains, a plains-type ecoregion in Kansas with a high proportion of cultivated agriculture, was correlated with elevated NH_4^+. The elevated NH_4^+ could result from farming practices or decomposition of organic matter during low-flow periods where in-stream N recycling, also likely to be higher in the summer, is elevated (Kalff 2001, Sliva and Williams 2001).

When the influence of physiographic factors was removed, the influence of LULC factors on in-stream nutrient concentrations also changed from the first LULC analysis. The effects of catchment-scale rangeland and reach-scale open water were minimized (Fig. 1.5B). The analysis still primarily represented a land use intensity gradient between catchment-scale urban LULC and reach-scale forest cover. However, a secondary vegetation cover type gradient became apparent, with relatively smaller influence from reach-scale urban and cultivated LULC. In the independent analysis, which did not factor out the confounding influence of physiographic predictors, catchment-scale rangeland and reach-scale open water most closely correlated with mean slope and catchment area. However, their minimal influence in the partial RDA suggests they do not explain additional information beyond that of physiography. In this partial RDA, catchment-scale urban LULC was most closely associated with higher TN and NO_3^- concentrations, consistent with the expected effect of urban LULC on nutrient concentrations (Paul and Meyer 2001). Interestingly, suspended particulate matter and seston C:N were associated with reach-scale cultivated land and reach-scale rangeland, and negatively correlated with catchment-scale forest cover. This pattern is substantially different than the relationship observed in the combined analysis (Fig. 1.4C) in which particulate matter and seston C:N were positively correlated with percent

cover of open water. This is consistent with the idea that forests retain more organic matter and suspended solids on the landscape (Kaplan et al. 2006). The lack of an association between these parameters and reach-scale forest cover suggests that the processes retaining these nutrients on the landscape operate at broad scales and that the effectiveness of local nutrient attenuation can be overwhelmed by catchment-level processes (Arango and Tank 2008, Filoso and Palmer 2011).

Here, the use of variance partitioning allowed us to understand the degree to which physiographic and LULC predictors individually influenced in-stream nutrient concentrations. Cumulatively, both physiographic and LULC characteristics explained 51% of the variation in nutrient concentrations in the watershed. That watershed conditions and land use impact aquatic nutrient dynamics is well established (Allan et al. 1997, Allan 2004, Dow et al. 2006, Johnson and Host 2010), however, it is surprising that half of the variability can be accounted for using metrics that may only change on decadal or greater time scales. Contrary to our expectations, when this explained variation is partitioned, physiographic predictors accounted for double the amount variability explained by LULC predictors (25% versus 12% of the total explained variation, respectively). However, despite this finding, and substantial overlap between the two sets of predictors (14%), our analysis suggested that the inclusion of both physiographic and LULC data sets was critical to understand the independent effects of both types of data. To our knowledge, the only other study to use similar variance partitioning techniques on an equivalently extensive nutrient dataset is Dow et al. (2006), which examined the watershed influence on the major ions in a ~5100 km^2 drinking water supply catchment in New York State where the datasets were partitioned into detailed LULC and the spatial distribution of geologic features. That study found substantial overlap between LULC and geology, with LULC explaining more of the variation in aquatic ion concentrations. In contrast to Dow et al. (2006), we found that patterns

of LULC had less control over nutrient chemistry than physiographic parameters and environmental gradients (which likely incorporate much of the geologic variation). The smaller geographic area and more intensive sampling of that study likely accounts for some of the higher proportion of variance explained, as well as the predominant influence of LULC predictors (Goldstein et al. 2007).

Conclusions

The primary goal with this study was to assess the degree to which relatively static measures of physiographic environmental conditions and patterns of LULC can predict nutrient conditions in a large-scale riverine system. Contrary to our first hypothesis, we found that over the large area of the Brazos River watershed, physiographic environmental gradients had a stronger influence over baseline water chemistry than patterns of LULC. However, as we predicted for our second hypothesis, the effect of analysis scale was highly dependent on the chosen response variable and category of predictor, with reach and catchment-scales having varying influences. Physiographic gradients and conditions appear to set a baseline context by which nutrient conditions are controlled in large lotic systems. Land use and land cover are highly correlated with physiography, yet still have significant independent influences on nutrient concentrations. This is important information for researchers designing management or restoration projects. Understanding that a stream reach sits in a larger landscape context can allow for the incorporation of more appropriate restoration measures, as well as more realistic expectations about the larger benefits of a given project. For example, riparian restoration is widely used to reduce in-stream nutrient concentrations, but the effect restoration will have depends on the nutrient in question, the steepness and size of the catchment, and whether the larger watershed is located in a predominantly agricultural or forested region. The use of physiographic predictors will be most beneficial in large systems

that span large areas, where environmental gradients can have stronger influences than land use (Goldstein et al. 2007), or smaller systems that have particularly steep environmental gradients (Malmqvist 2002). Obviously, more dynamic measures of environmental conditions (e.g., high resolution rainfall monitoring and watershed-wide hydrologic modeling) would explain an additional portion of the in-stream nutrient concentrations, but are potentially cost prohibitive on such a large scale. More proximate measures of precipitation, geology, or agricultural fertilizer application rates would also likely explain additional variation in the nutrient loading in the Brazos watershed, but what these results highlight is the legacy that largely static physiographic conditions and long term patterns of climate and land use have on aquatic systems.

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Table 1.S1 Sampling locations, physiographic, and land use predictors used in the present study. Abbreviations are consistent with Table 1.1. The number after the watershed corresponds to sampling order, in an upstream/upwatershed direction. Lat = lattitude, Long = longitude, Ecoregion = US EPA Level III ecoregion, MAP = mean annual precipitation (1961-1990), Max Slope = maximum slope, s.d. Slope = sandard deviation of slope. The number after the scaleable variables is consistent with the text. 1 = reach-scale, 2 = riparian-scale, 3 = catchment-scale

Site Code	Site Name/Description	Physiographic Predictors														
		Lat (decimal°)	Long (decimal°)	Ecoregion	MAP (cm)	Catchment Area (km^2)	Stream Density (km/km^2)	Max Slope 1 (% grade)	Mean Slope 1 (% grade)	s.d. Slope 1 (% grade)	Max Slope 2 (% grade)	Mean Slope 2 (% grade)	s.d. Slope 2 (% grade)	Max Slope 3 (% grade)	Mean Slope 3 (% grade)	s.d. Slope 3 (% grade)
Yegua Creek watershed																
YG1	Yegua Creek at SH50	30.3681	-96.3432	ECTP	104	3343.35	0.599	8	1.6	1.6	26	1.2	1.3	38	1.4	1.3
YG2	Yegua Creek at SH36	30.3215	-96.5073	ECTP	99	2566.59	0.605	13	1.9	2.7	26	1.2	1.3	38	1.4	1.3
YG3	West Yegua Creek at SH21	30.2913	-96.9605	ECTP	94	264.05	0.613	6	1.1	1.1	26	1.3	1.4	26	1.5	1.3
Little River watershed																
LR1	Little River at CR264	30.8254	-96.7436	ECTP	94	19532.49	0.527	21	4.8	3.7	40	2.1	2.5	40	1.8	2.1
LR2	Big Elm Creek at US77	30.9030	-96.9791	TBPR	89	812.80	0.530	5	0.9	1.1	22	1.2	1.4	22	1.0	1.1
LR3	San Gabriel at CR428	30.6944	-97.2788	TBPR	89	1884.09	0.544	12	3.9	2.7	37	2.0	2.3	40	1.4	1.7
LR4	San Gabriel at Shady RV campground	30.6373	-97.5725	TBPR	89	1466.00	0.533	12	3.1	2.2	37	2.1	2.5	40	1.6	1.8
LR5	North San Gabriel at US183	30.7031	-97.8773	EDPL	84	515.63	0.530	11	3.1	2.2	26	2.1	2.3	26	1.5	1.5
LR6	South San Gabriel at US183	30.6207	-97.8609	EDPL	84	254.09	0.596	30	4.9	4.7	30	2.3	2.6	30	1.6	1.6
LR7	Brushy Creek at CR685	30.5261	-97.5665	TBPR	89	392.98	0.612	21	1.8	2.3	24	1.5	1.8	24	1.2	1.3
Central Brazos subbasins																
CW1	Old River at FM444	30.4040	-96.3141	ECTP	104	176.17	0.877	20	4.6	3.7	20	0.7	1.4	21	0.6	1.1
CW2	Thompsons Creek at 1688	30.6009	-96.4435	ECTP	99	125.66	0.882	7	1.3	1.3	14	1.2	1.2	14	1.1	1.0
CW3	Little Brazos River at SH21	30.6409	-96.5206	ECTP	99	746.72	0.599	11	2.5	2.4	13	1.4	1.7	20	1.3	1.5
CW4	Big Creek at SH6	31.2568	-96.8598	TBPR	94	780.62	0.666	3	0.4	0.8	10	0.9	1.0	14	0.7	0.9
CW5	Deer Creek at SH935	31.2648	-97.0320	TBPR	89	228.45	0.505	10	2.3	1.9	14	1.5	1.5	19	1.4	1.3
CW6	Tehuacana Creek at FM2491	31.5640	-97.0481	TBPR	84	476.37	0.535	3	0.5	0.7	12	1.2	1.3	13	1.0	1.1
Navasota River watershed																
NR1	Navasota River at SH6	30.4183	-96.1065	TBPR	104	5625.69	0.626	10	4.1	2.5	26	1.1	1.4	26	1.2	1.3
NR2	Navasota River at Sulphur Springs Rd.	30.5707	-96.1665	ECTP	104	4834.33	0.607	8	0.5	0.9	26	1.1	1.4	26	1.2	1.3
NR3	Navasota River at CR162	30.7204	-96.1677	ECTP	104	4286.08	0.596	2	0.1	0.3	26	1.2	1.4	26	1.2	1.3
NR4	Navasota River at US79	31.1695	-96.2986	ECTP	99	2405.98	0.586	3	0.8	0.9	21	1.1	1.3	21	1.1	1.2
NR5	Navasota River at SH164	31.5125	-96.4511	ECTP	99	792.22	0.581	4	0.9	1.0	13	0.9	1.0	20	0.8	0.9
NR6	Navasota River at SH73	31.7018	-96.7223	TBPR	94	143.00	0.685	2	0.5	0.6	7	0.8	0.9	9	0.8	0.7
Lampasas River watershed																
LM1	Lampasas River at IH35	31.0019	-97.4919	TBPR	84	3376.11	0.499	23	5.3	4.1	35	2.9	2.9	35	2.5	2.4
LM2	Lampasas River at SH195	30.9724	-97.7782	EDPL	84	3049.18	0.503	28	5.2	4.1	35	2.8	2.8	35	2.4	2.3
LM3	Lampasas River at US190	31.0794	-98.0159	EDPL	79	2083.56	0.499	14	3.4	2.3	31	2.6	2.6	31	2.4	2.2
Lower Brazos subbasins																
LB1	Big Creek at Brazos Bend State Park	29.3784	-95.6024	WGCP	114	414.17	0.847	10	3.1	2.5	10	0.5	0.8	10	0.1	0.5
LB2	Bullhead Bayou at SH99	29.6066	-95.6866	WGCP	114	12.44	1.948	4	0.7	0.8	5	0.3	0.7	5	0.2	0.4
LB3	Allens Creek at Mixville Rd.	29.7039	-96.1290	WGCP	104	25.80	0.719	3	1.0	0.8	3	0.5	0.7	3	0.3	0.5
LB4	Irons Creek at CR1458	29.8268	-96.0364	WGCP	104	142.31	0.590	4	0.2	0.5	9	0.7	0.9	9	0.3	0.6
LB5	Mill Creek at CR331	29.8695	-96.1550	ECTP	104	1005.71	0.572	10	2.9	2.4	12	1.7	1.6	13	1.9	1.4
LB6	Clear Creek at CR3346	30.0544	-96.0580	ECTP	104	133.09	0.629	6	1.1	1.1	9	1.2	1.2	9	0.9	0.9
LB7	Caney Creek at CR1456	30.0621	-96.2090	TBPR	104	114.30	0.541	7	0.9	1.1	9	1.6	1.5	12	2.0	1.4
LB8	New Year Creek at CR2447	30.1657	-96.2233	TBPR	104	428.87	0.534	9	1.9	1.6	12	1.4	1.4	12	1.9	1.3
Mainstem sites																
MS1	Mainstem Brazos at FM1093	29.6712	-96.0212	WGCP	109	116983.96	0.413	21	5.3	3.9	44	2.0	2.7	46	1.4	2.2
MS2	Mainstem Brazos at SH159	30.0439	-96.1099	TBPR	104	114857.19	0.410	13	3.9	3.4	44	2.0	2.7	46	1.4	2.2
MS3	Mainstem Brazos at SH21	30.6278	-96.5440	TBPR	99	102384.78	0.386	16	3.9	3.4	44	2.1	2.9	46	1.4	2.3
MS4	Mainstem Brazos at FM712	31.2468	-96.9207	ECTP	94	79562.76	0.344	3	0.6	1.0	44	2.2	3.1	46	1.3	2.3

Table 1.S1 Continued

Land Use/Land Cover Predictors																	
Urban 1 (% cover)	Cultivated 1 (% cover)	Range 1 (% cover)	Forest 1 (% cover)	Water 1 (% cover)	Wetlands 1 (% cover)	Urban 2 (% cover)	Cultivated 2 (% cover)	Range 2 (% cover)	Forest 2 (% cover)	Water 2 (% cover)	Wetlands 2 (% cover)	Urban 3 (% cover)	Cultivated 3 (% cover)	Range 3 (% cover)	Forest 3 (% cover)	Water 3 (% cover)	Wetlands 3 (% cover)
2.10	41.09	8.67	1.05	0.00	47.09	2.62	23.63	14.20	20.04	2.60	36.92	5.45	37.58	20.73	27.68	1.61	6.96
4.56	8.70	6.05	10.83	13.38	56.48	2.74	22.93	14.41	18.89	3.17	37.86	5.42	37.30	21.45	26.66	1.99	7.18
3.04	33.68	6.32	3.16	0.00	53.80	2.47	26.89	14.22	12.93	1.04	42.45	4.41	43.19	20.11	25.32	0.28	6.69
1.67	7.86	6.88	22.00	13.36	48.23	5.11	11.51	47.09	23.78	2.98	9.53	7.79	17.12	55.42	17.10	0.90	1.67
6.38	20.89	17.22	48.80	0.00	6.70	4.05	32.88	31.69	10.55	2.26	18.58	7.50	51.66	32.90	4.16	0.58	3.20
9.06	10.19	9.22	3.40	0.00	68.12	6.65	6.37	46.48	28.31	4.22	7.96	9.52	11.71	52.58	23.29	1.38	1.53
7.65	9.47	26.67	25.28	4.60	26.32	6.50	0.93	50.04	35.60	2.06	4.87	9.84	2.27	57.82	28.89	0.49	0.69
4.33	0.00	58.96	29.85	3.43	3.43	2.38	0.08	58.23	35.43	0.20	3.68	3.51	0.06	68.31	27.65	0.06	0.42
12.61	0.00	20.48	62.67	3.52	0.73	4.86	0.07	42.02	51.01	0.80	1.24	8.20	0.03	51.32	40.01	0.14	0.31
36.81	7.49	25.19	6.73	0.00	23.78	30.10	1.21	29.47	23.33	3.38	12.50	43.53	2.79	31.85	19.22	0.63	1.98
1.61	13.85	0.00	1.93	12.72	69.89	3.95	61.89	8.35	10.36	1.02	14.42	4.06	65.93	11.56	13.82	0.28	4.35
1.76	39.87	25.11	0.33	0.00	32.93	9.27	21.64	15.39	16.46	1.10	36.13	19.51	35.25	19.36	16.11	1.32	8.45
2.81	44.84	4.06	0.31	0.00	47.97	3.69	29.90	11.58	24.81	0.64	29.37	6.45	43.47	15.32	29.09	0.53	5.14
4.61	52.44	18.54	22.98	0.62	0.80	3.14	31.79	33.31	19.87	1.02	10.88	6.24	52.81	31.71	6.80	0.37	2.08
4.29	53.58	23.93	4.50	0.00	13.70	5.01	25.10	41.60	4.88	0.47	22.93	7.54	45.36	39.60	2.66	0.30	4.54
1.43	22.05	23.93	12.24	11.47	28.89	3.13	36.68	30.27	8.65	3.11	18.16	9.56	55.52	26.39	4.25	0.77	3.52
4.19	14.50	4.04	9.87	11.96	55.46	3.87	23.97	14.98	26.33	4.32	26.53	7.10	39.04	21.70	22.76	1.82	7.58
0.24	3.46	0.16	1.49	0.94	93.71	3.93	21.82	16.53	29.18	4.06	24.48	7.08	37.31	23.59	23.65	1.74	6.63
2.56	2.47	3.44	0.70	0.00	90.84	2.64	22.01	17.37	30.23	4.48	23.28	5.66	37.06	24.84	24.47	1.88	6.09
2.95	0.00	5.08	20.49	0.00	71.48	2.85	20.89	20.96	32.15	5.88	17.27	6.41	36.86	29.85	20.13	2.69	4.07
1.35	22.58	61.07	12.90	0.00	2.10	2.61	21.34	29.24	38.19	3.17	5.45	5.92	35.07	43.32	13.21	1.00	1.48
3.23	9.13	10.84	67.11	0.00	9.70	2.52	30.98	22.52	42.45	0.04	1.49	5.00	45.93	38.28	10.23	0.14	0.42
8.02	2.90	4.23	22.05	1.56	61.25	2.69	1.08	58.79	31.57	2.74	3.13	4.34	1.42	70.60	22.19	0.98	0.47
5.26	0.00	41.05	29.44	5.55	18.70	2.49	1.07	61.03	31.55	1.17	2.69	3.60	1.46	73.51	20.88	0.17	0.37
3.50	0.00	57.23	12.08	13.20	13.99	2.42	1.39	66.59	26.34	1.04	2.23	3.09	1.86	79.12	15.46	0.17	0.30
0.00	9.05	0.00	0.00	0.00	90.95	5.37	66.35	6.26	3.81	0.78	17.43	7.15	75.52	6.48	1.86	0.60	8.39
7.63	91.97	0.40	0.00	0.00	0.00	29.04	67.95	1.83	1.19	0.00	0.00	51.64	44.26	2.78	0.49	0.10	0.73
3.40	58.74	18.93	2.91	0.00	16.02	15.27	42.09	12.73	5.34	0.00	24.57	22.74	55.69	11.45	6.62	0.12	3.38
4.34	47.09	7.80	3.95	0.00	36.82	4.51	55.73	5.38	2.64	0.33	31.41	4.58	73.51	6.91	5.31	0.31	9.38
1.81	28.40	4.36	11.93	0.00	53.50	3.34	39.80	5.77	9.59	0.26	41.24	5.97	56.00	11.08	19.39	0.30	7.26
1.98	16.39	0.73	1.36	0.00	79.54	4.68	40.82	4.29	3.56	0.68	45.97	10.32	55.75	8.15	15.21	0.44	10.13
5.36	26.90	8.36	2.25	0.00	57.13	3.07	51.34	6.77	3.61	0.84	34.37	4.54	58.35	18.31	12.91	0.68	5.22
2.88	43.94	2.44	6.13	0.00	44.61	4.98	52.05	8.04	3.22	0.61	31.11	10.69	59.03	12.80	12.25	0.40	4.83
3.62	1.07	3.84	24.95	43.71	22.81	4.17	13.27	49.17	18.88	3.70	10.80	6.46	30.93	49.09	10.86	0.87	1.78
4.09	1.84	0.82	0.00	45.81	47.44	4.21	12.93	50.57	18.72	3.64	9.93	6.46	30.38	49.83	10.79	0.87	1.67
2.61	34.47	5.78	5.10	23.02	29.02	4.28	9.53	57.43	18.92	3.71	6.13	6.42	28.94	53.56	9.30	0.79	0.98
6.31	5.91	1.43	5.09	69.65	11.61	4.06	7.35	64.57	16.10	3.93	3.99	6.11	30.83	54.37	7.18	0.78	0.73

Table 1.S2 Annual average of water quality parameters for the Brazos River (Mean ± s.e), collected during 2008-2009. Site Codes are consistent with Table1.S1. TP = total phosporous, TN = total nitrogen, SRP = soluble reactive phosphorous, PP = particulate phosphorous, NO3- = nitrate, NH4+ = ammonium, PN = particulate N, PC = particluate carbon, DOC = dissolved organic carbon, NVSS = non-volatile suspended particles, SPOM = suspended particulate organic matter, C:N = seston molar carbon to nitrogen ratio, C:P = seston molar carbon to phosphorous ratio, N:P = seston molar nitrogen to phosphorous ratio, Chl a = chlorophyll a, Temp = temperature, DO = dissolved oxygen.

Site Code	TP (µg/L)	TN (µg/L)	SRP (µg/L)	PP (µg/L)	NO_3^- (µg/L)	NH_4^+ (µg/L)	PN (µg/L)	PC (mg/L)	DOC (mg/L)	NVSS (mg/L)	SPOM (mg/L)	C:N molar	C:P molar	N:P molar	Chl *a* (µg/L)	Temp (°C)	DO (mg/L)	pH
Yegua Creek watershed																		
YG1	142 ± 54	587 ± 106	89 ± 33	98 ± 28	190 ± 81	54 ± 6	662 ± 145	3.2 ± 0.7	9.7 ± 1.4	90 ± 31	18.1 ± 5.2	5.8 ± 0.6	95.9 ± 9.7	17.6 ± 2.8	5.1 ± 1.1	21 ± 5	6.6 ± 1.5	7.7 ± 0.2
YG2	103 ± 8	824 ± 176	37 ± 11	107 ± 13	100 ± 20	51 ± 8	772 ± 154	4.1 ± 0.4	6.9 ± 0.5	23 ± 2	15.1 ± 1.6	5.3 ± 0.3	103.8 ± 13.4	17.0 ± 3.9	25.9 ± 4.8	22 ± 4	5.7 ± 1.0	7.8 ± 0.2
YG3	81 ± 14	630 ± 85	60 ± 14	83 ± 11	171 ± 63	95 ± 29	544 ± 111	2.4 ± 0.3	9.7 ± 0.6	13 ± 3	9.5 ± 1.6	5.6 ± 0.6	74.7 ± 6.2	14.1 ± 1.6	8.8 ± 1.6	20 ± 4	6.8 ± 2.8	7.6 ± 0.3
Little River watershed																		
LR1	206 ± 41	2,590 ± 406	353 ± 172	152 ± 50	2,785 ± 756	130 ± 34	789 ± 221	7.9 ± 2.8	4.0 ± 0.3	84 ± 46	26.1 ± 11.3	10.8 ± 2.8	132.1 ± 20.6	14.7 ± 2.6	21.8 ± 8.4	18 ± 6	9.3 ± 1.5	7.8 ± 0.1
LR2	111 ± 26	5,397 ± 3,216	102 ± 23	62 ± 13	5,311 ± 3,300	78 ± 16	308 ± 84	2.2 ± 0.5	8.3 ± 1.5	30 ± 7	10.7 ± 2.9	9.4 ± 2.5	89.8 ± 8.3	12.0 ± 1.9	2.4 ± 0.6	17 ± 6	8.2 ± 1.5	7.8 ± 0.0
LR3	26 ± 5	1,489 ± 330	12 ± 4	45 ± 15	1,718 ± 237	37 ± 5	308 ± 101	2.1 ± 0.7	2.3 ± 0.9	15 ± 7	6.6 ± 2.7	8.4 ± 1.2	144.3 ± 34.6	19.2 ± 5.6	13.1 ± 9.5	18 ± 5	9.0 ± 1.0	7.8 ± 0.2
LR4	338 ± 103	4,682 ± 1,333	763 ± 396	31 ± 8	6,428 ± 2,202	69 ± 6	188 ± 29	0.9 ± 0.1	3.5 ± 1.3	1 ± 0.2	2.7 ± 0.5	6.1 ± 0.7	121.2 ± 51.5	23.2 ± 10.8	5.3 ± 0.7	21 ± 6	14.5 ± 3.5	8.8 ± 0.4
LR5	20 ± 5	742 ± 200	18 ± 7	21 ± 5	202 ± 54	50 ± 10	154 ± 46	1.1 ± 0.3	14.9 ± 7.3	2 ± 1	2.7 ± 0.8	8.6 ± 1.3	254.5 ± 131.4	40.2 ± 24.3	2.1 ± 0.7	19 ± 4	7.9 ± 1.3	8.5 ± 0.2
LR6	7 ± 1	233 ± 70	8 ± 3	8 ± 4	270 ± 75	35 ± 4	78 ± 17	0.4 ± 0.1	2.0 ± 0.5	0.1 ± 0.2	1.1 ± 0.1	6.5 ± 1.7	111.3 ± 16.5	22.5 ± 7.2	0.6 ± 0.1	20 ± 7	11.9 ± 2.0	8.1 ± 0.0
LR7	991 ± 83	7,443 ± 1,716	1,590 ± 211	63 ± 18	7,752 ± 1,412	68 ± 6	216 ± 27	1.1 ± 0.1	4.8 ± 1.0	2 ± 1	3.5 ± 0.8	6.4 ± 1.1	74.3 ± 25.8	16.1 ± 7.6	3.4 ± 0.7	20 ± 4	8.5 ± 1.5	7.9 ± 0.2
Central Brazos subbasins																		
CW1	212 ± 59	641 ± 105	322 ± 130	68 ± 19	164 ± 70	49 ± 7	378 ± 121	1.6 ± 0.3	6.1 ± 1.1	17 ± 7	7.9 ± 2.5	6.1 ± 1.1	75.0 ± 15.3	13.8 ± 3.3	4.0 ± 0.8	20 ± 5	3.9 ± 0.5	7.7 ± 0.0
CW2	4,387 ± 236	27,529 ± 3,408	4,348 ± 926	217 ± 80	42,487 ± 6,549	38 ± 7	1,007 ± 284	3.9 ± 1.0	3.4 ± 1.3	155 ± 72	27.8 ± 11.3	4.7 ± 0.5	69.5 ± 22.2	14.4 ± 3.4	7.1 ± 1.9	20 ± 5	7.9 ± 1.6	8.3 ± 0.1
CW3	211 ± 38	1,116 ± 178	235 ± 101	105 ± 54	616 ± 106	40 ± 5	374 ± 154	2.3 ± 1.0	2.9 ± 0.6	45 ± 27	12.6 ± 6.3	7.2 ± 0.9	77.2 ± 15.6	11.6 ± 2.2	4.5 ± 1.1	20 ± 6	8.5 ± 1.5	8.0 ± 0.1
CW4	148 ± 23	1,081 ± 155	39 ± 8	170 ± 48	284 ± 137	42 ± 4	857 ± 286	6.0 ± 2.1	8.5 ± 1.3	110 ± 43	26.3 ± 8.2	7.4 ± 1.2	89.0 ± 9.8	12.6 ± 4.4	24.2 ± 12.0	19 ± 6	7.3 ± 1.1	8.1 ± 0.1
CW5	306 ± 167	1,113 ± 239	494 ± 305	85 ± 36	403 ± 128	64 ± 18	401 ± 106	2.3 ± 0.2	6.6 ± 4.6	15 ± 2	8.2 ± 1.2	8.7 ± 1.7	116.9 ± 30.0	15.1 ± 4.7	17.6 ± 9.4	21 ± 7	10.0 ± 2.5	8.3 ± 0.1
CW6	4,254 ± 1,294	3,726 ± 1,137	4,232 ± 1,623	159 ± 52	2,901 ± 1,227	34 ± 3	547 ± 210	4.1 ± 1.8	6.9 ± 2.3	122 ± 67	25.1 ± 12.6	9.0 ± 2.2	41.6 ± 8.2	5.2 ± 0.9	7.3 ± 1.4	20 ± 6	10.7 ± 1.3	8.3 ± 0.2
Navasota River watershed																		
NR1	531 ± 86	3,241 ± 541	830 ± 289	91 ± 8	3,052 ± 599	42 ± 3	446 ± 88	1.7 ± 0.1	6.0 ± 0.4	42 ± 3	9.6 ± 2.7	5.0 ± 0.7	51.6 ± 8.2	13.1 ± 4.6	3.5 ± 0.6	22 ± 6	7.2 ± 1.7	8.0 ± 0.1
NR2	559 ± 78	2,808 ± 429	678 ± 12	91 ± 12	2,451 ± 217	59 ± 6	558 ± 100	2.2 ± 0.2	5.9 ± 0.7	41 ± 3	9.9 ± 1.5	5.6 ± 1.3	67.9 ± 9.3	16.5 ± 4.8	4.2 ± 0.7	21 ± 6	7.6 ± 1.9	7.8 ± 0.1
NR3	59 ± 6	546 ± 98	39 ± 4	39 ± 10	212 ± 67	27 ± 4	285 ± 42	1.1 ± 0.1	4.9 ± 0.3	15 ± 5	5.2 ± 1.8	5.2 ± 1.3	86.9 ± 15.3	23.4 ± 7.1	2.7 ± 0.4	21 ± 6	8.2 ± 2.0	7.6 ± 0.1
NR4	68 ± 11	566 ± 77	28 ± 4	85 ± 21	345 ± 67	28 ± 5	488 ± 90	2.7 ± 0.7	4.8 ± 0.2	63 ± 28	14.7 ± 5.6	6.3 ± 1.2	80.6 ± 7.5	14.3 ± 2.1	13.9 ± 2.9	20 ± 5	8.3 ± 1.6	7.5 ± 0.1
NR5	251 ± 66	856 ± 159	489 ± 273	133 ± 16	609 ± 299	72 ± 8	741 ± 102	3.9 ± 0.4	6.9 ± 0.2	41 ± 14	13.9 ± 2.9	6.4 ± 0.9	76.0 ± 3.9	13.1 ± 2.1	29.7 ± 9.1	23 ± 6	9.5 ± 3.4	8.2 ± 0.3
NR6	245 ± 44	1,085 ± 130	290 ± 91	122 ± 37	194 ± 89	50 ± 7	567 ± 133	2.6 ± 0.6	14.0 ± 1.9	27 ± 13	10.8 ± 3.6	5.7 ± 0.8	68.3 ± 10.9	12.3 ± 1.9	10.6 ± 4.8	20 ± 4	6.4 ± 0.8	7.7 ± 0.1
Lampasas River watershed																		
LM1	50 ± 18	710 ± 59	45 ± 22	20 ± 7	547 ± 50	34 ± 3	140 ± 21	0.9 ± 0.3	2.0 ± 0.1	6 ± 3	3.5 ± 1.1	8.0 ± 1.9	138.2 ± 20.1	28.9 ± 12.2	3.4 ± 1.5	17 ± 6	7.8 ± 2.0	7.9 ± 0.2
LM2	59 ± 15	332 ± 99	53 ± 19	56 ± 27	155 ± 89	55 ± 7	361 ± 170	4.4 ± 1.9	2.9 ± 0.6	39 ± 21	14.5 ± 7.0	13.9 ± 3.3	359.3 ± 106.4	61.6 ± 45.7	2.0 ± 0.5	17 ± 6	8.8 ± 2.1	8.1 ± 0.1
LM3	72 ± 10	687 ± 128	83 ± 10	34 ± 14	257 ± 108	57 ± 9	220 ± 58	2.1 ± 0.7	3.1 ± 0.7	16 ± 8	7.7 ± 3.4	11.4 ± 2.6	187.9 ± 29.6	21.1 ± 4.9	2.7 ± 0.8	19 ± 6	11.4 ± 2.3	8.3 ± 0.1
Lower Brazos subbasins																		
LB1	480 ± 284	1,273 ± 272	248 ± 128	63 ± 11	636 ± 193	120 ± 3	293 ± 46	1.6 ± 0.2	6.6 ± 0.6	24 ± 5	7.8 ± 1.4	7.2 ± 1.4	72.6 ± 6.8	11.7 ± 2.1	9.8 ± 3.2	25 ± 4	8.4 ± 1.4	8.1 ± 0.2
LB2	5,134 ± 442	6,169 ± 1,565	4,203 ± 818	579 ± 94	6,100 ± 1,874	156 ± 44	502 ± 79	2.7 ± 0.3	3.9 ± 0.2	47 ± 5	11.5 ± 2.0	6.9 ± 1.1	12.5 ± 0.9	2.0 ± 0.3	10.9 ± 2.0	26 ± 2	8.0 ± 3.3	8.1 ± 0.3
LB3	2,055 ± 331	10,695 ± 952	2,674 ± 591	65 ± 12	9,129 ± 635	50 ± 9	206 ± 31	0.9 ± 0.1	4.8 ± 0.3	7 ± 2	3.9 ± 1.0	5.9 ± 1.2	41.8 ± 7.7	8.5 ± 2.6	1.2 ± 0.1	23 ± 2	6.9 ± 1.0	7.6 ± 0.2
LB4	210 ± 71	548 ± 82	104 ± 30	96 ± 25	76 ± 14	57 ± 6	454 ± 63	2.3 ± 0.4	6.6 ± 0.6	19 ± 11	8.5 ± 2.8	6.0 ± 0.8	66.9 ± 5.9	12.1 ± 1.8	20.3 ± 3.8	23 ± 4	6.8 ± 1.8	7.3 ± 0.1
LB5	177 ± 27	583 ± 113	172 ± 20	45 ± 16	191 ± 52	34 ± 5	234 ± 46	1.4 ± 0.4	4.0 ± 0.7	11 ± 10	7.9 ± 3.3	7.5 ± 1.9	93.0 ± 13.8	18.6 ± 5.6	3.0 ± 0.5	28 ± 4	10.2 ± 1.5	8.3 ± 0.2
LB6	309 ± 76	1,709 ± 149	323 ± 48	60 ± 4	1,538 ± 97	34 ± 8	150 ± 26	0.7 ± 0.1	3.7 ± 0.1	4 ± 1	3.3 ± 0.7	7.1 ± 2.0	30.0 ± 1.3	6.0 ± 1.3	1.4 ± 0.4	23 ± 2	6.4 ± 0.1	7.6 ± 0.1
LB7	177 ± 49	400 ± 77	155 ± 36	35 ± 11	135 ± 59	66 ± 9	300 ± 85	1.9 ± 1.0	4.3 ± 0.5	28 ± 20	8.0 ± 4.4	7.0 ± 1.5	120.9 ± 20.8	21.1 ± 5.7	8.9 ± 3.6	23 ± 2	5.0 ± 0.9	7.8 ± 0.2
LB8	331 ± 19	2,293 ± 651	439 ± 77	33 ± 2	1,783 ± 609	66 ± 5	208 ± 28	1.2 ± 0.1	5.7 ± 0.2	14 ± 1	5.1 ± 0.7	7.3 ± 1.3	90.2 ± 3.8	14.3 ± 2.5	3.1 ± 0.3	23 ± 3	6.4 ± 1.0	8.0 ± 0.2
Mainstem sites																		
MS1	353 ± 88	1,233 ± 109	157 ± 63	628 ± 176	741 ± 217	38 ± 4	1,411 ± 254	18.7 ± 6.2	4.6 ± 0.3	541 ± 174	102.0 ± 30.2	20.3 ± 7.4	126.4 ± 39.2	14.9 ± 7.2	47.4 ± 25.9	24 ± 3	8.2 ± 3.1	8.0 ± 0.2
MS2	310 ± 65	1,409 ± 81	152 ± 56	338 ± 101	894 ± 163	56 ± 7	2,151 ± 444	21.5 ± 5.3	5.1 ± 0.4	272 ± 104	61.2 ± 23.2	12.1 ± 3.6	179.8 ± 45.3	19.0 ± 4.8	45.8 ± 24.3	24 ± 3	7.2 ± 2.1	7.8 ± 0.1
MS3	163 ± 49	1,520 ± 169	49 ± 23	126 ± 44	827 ± 252	31 ± 8	708 ± 169	5.3 ± 1.2	3.0 ± 1.0	69 ± 33	21.7 ± 6.8	8.9 ± 1.4	132.3 ± 26.1	16.9 ± 4.3	27.8 ± 5.2	20 ± 6	9.4 ± 1.5	8.1 ± 0.1
MS4	64 ± 7	1,121 ± 159	29 ± 17	84 ± 19	631 ± 190	32 ± 7	563 ± 140	3.7 ± 0.9	3.4 ± 1.0	19 ± 7	13.4 ± 3.7	7.5 ± 1.0	113.2 ± 17.4	16.5 ± 3.0	18.8 ± 4.7	20 ± 7	14.6 ± 3.5	8.5 ± 0.3

CHAPTER II

CONCORDANCE AND SPATIAL AUTOCORRELATION BETWEEN PHYSICOCHEMICAL CONDITIONS, MACROINVERTEBRATE, AND FISH COMMUNITIES IN A GULF SLOPE RIVER ECOSYSTEM

Abstract

We assessed the patterns of macroinvertebrate and fish community diversity and distribution in the Brazos River (TX). Additionally, we examined the interactions between the biotic communities and spatial arrangement on the landscape as well as physicochemical environmental conditions this large Gulf Slope river system. At the scale investigated, we found that macroinvertebrate and fish community compositions and physicochemical condition were all correlated, and appear to be largely influenced by broad-scale environmental gradients. We found common trends in all three datasets using multiple complimentary analysis techniques, however the fish community was additionally influenced by local community interactions. The composition of the macroinvertebrate and fish communities changed at sites in an upstream-to-downstream manner, and the interactions were strongest in the spring and summer. However, the utility of using one community to predict the other was limited. This study adds to the growing body of work indicating that surrogate species or species groups in monitoring and conservation programs should be chosen with caution. Additionally, disentangling the roles of exogenous and endogenous influences on community distributions and interactions is important for understanding the response communities will have to changes in environmental conditions.

Introduction

Ecosystems are composed of complex networks of organisms that interact with each other and their abiotic environment. Understanding broad patterns in the ways in which different groups of species interact with each other and their environment and how species are distributed in ecosystems are the central goals of ecology (Pianka 1988, Bahn and McGill 2007) and an improved understanding of these relationships would enhance our ability to conserve species and restore ecosystems (Heino 2010). Understanding the common patterns of species richness, diversity, or assemblage structure between different taxa (what we refer to as concordance, but also commonly called congruence) and their responses to environmental gradients has the potential to allow ecologists and environmental scientists to better predict system-wide outcomes of stressors such as global climate change (Heino 2010). For example, the composition of fish communities in stream ecosystems is influenced by external environmental gradients and in-stream habitat conditions (Paavola et al. 2006); however, the densities and relative abundances of the invertebrate prey community may also exert a bottom-up control on fish community structure (Jackson and Harvey 1993). In turn, changes in the fish community can exert pressure on the invertebrate community, which also influenced by environmental conditions (Jackson and Harvey 1993, Heino 2010).

Traditionally, ecologists have examined how groups of species interact with each other and their environment with sets of a limited number of taxa and not entire communities (Paavola et al. 2006). The practical application of this perspective has led ecologists to often use specific groups of organisms to assess community-wide patterns in biodiversity and ecosystem-level health (e.g. Morley and Karr 2002). One of the assumptions of this method for ecological and conservation studies is that there is some degree of concordance between taxa and community assemblages (*sensu* Jackson and Harvey 1993). Although the use

of a limited number of organisms, taxonomic or functional groups to infer system-wide patterns in diversity and ecosystem health is widely used in ecological and bio-assessment studies, the broad applicability of this method for prediction can be problematic (Heino 2010). There is high variability in diversity patterns for different taxonomic groups (Paavola et al. 2006) and the degree of correlation between groups are inconsistent (Sánchez-Fernández et al. 2006, Heino 2010). Some of the methods commonly used to assess community concordance rely on similarity or distance matrices, which simplify the community information (Gioria et al. 2011, Padial et al. 2012). Methods that use the full community matrix rather than measures of resemblance have the potential to better elucidate species concordance patterns (ter Braak and Schaffers 2004, Gioria et al. 2011).

Many of the interactions between taxonomic groups and environmental conditions have spatial and temporal patterns that can influence the concordance between communities (Wilkinson and Edds 2001, Grenouillet et al. 2008, Padial et al. 2012). Spatial influences can come from two sources: induced spatial dependence from common responses to environmental gradients (exogenous) and inherent spatial autocorrelation from local biotic and community interactions (endogenous; Fortin and Dale 2005). For example, a stream community may exhibit patterns of species abundance that are driven by longitudinal position along a stream-course (exogenous influences; Vannote et al. 1980, Grenouillet et al. 2008) or the community may exhibit spatial patterns that are driven by biotic interactions such as predator-prey or competition dynamics (endogenous influences; Paavola et al. 2003, Padial et al. 2012). The importance of accounting for spatial autocorrelation has become recognized and researchers are more frequently accounting for these patterns (Wilkinson and Edds 2001, Legendre et al. 2002, Grenouillet et al. 2008). Whether patterns seen in nature are caused by exogenous or endogenous factors and researcher's ability to parse the differences has large

implications on our ability to understand and correctly interpret ecological data (Bahn and McGill 2007, Currie 2007). Additionally, conservation scientists and practitioners often rely on indicator species or surrogate groups to assess ecosystem health (Padial et al. 2012). Many conservation programs rely on the ability of one species (or group) to predict another, and it is important to know if the patterns seen are from exogenous or endogenous factors, as the mechanisms under each scenario would be different (Currie 2007, Grenouillet et al. 2008, Padial et al. 2012). Unfortunately, separating the effects of exogenous and endogenous spatial autocorrelation is difficult and they are often combined into a blanket assessment of spatial autocorrelation (Fortin and Dale 2005, Grenouillet et al. 2008). Through the use of multiple analysis methods researchers can begin to separate exogenous- and endogenously-influenced patterns of community concordance begin separating the roles of environment, space, and trophic interactions in determining community distributions and abundance (Currie 2007, Gioria et al. 2011, Padial et al. 2012).

In the study presented here, we examined the patterns of diversity and community concordance between macroinvertebrate and fish communities in a large, complex river drainage, and how organismal diversity and community concordance are related to physicochemical parameters. We assessed the patterns of spatial autocorrelation in the physicochemical, macroinvertebrate, and fish communities to evaluate whether the community patterns were more likely from exogenous or endogenous influences. Additionally, we assessed temporal changes in these relationships by using data collected over the course of a one-year period. Finally, we assessed the ability of one community to predict the structure of the other. We used macroinvertebrate and fish abundance data, along with physicochemical habitat and environmental data collected concurrently from the lower Brazos River watershed (Texas, USA). We predicted that there would be large-scale spatial concordance patterns in the species data, and that these patterns

would indicate primarily physiographic and habitat controls over community structure. Previous studies indicate that spatial variation in the fish community structure (Labay 2010), macroinvertebrate community structure (Lash 2011), and water chemistry (Becker et al. *in revision*) are all strongly influenced by large-scale variation in ecoregion type, mean annual rainfall, and land-use intensity in this portion of the Brazos River watershed. Thus, we expected that the patterns of spatial autocorrelation would be indicative of nutrients and biotic communities responding to these gradients, implying largely exogenous influences.

Methods

Study Area

The Brazos River spans a distance of 2060 river km from its source near the Texas – New Mexico border to the Gulf of Mexico, and is the 11th longest river in the United States. The watershed is ~116,000 km^2, and spans eight distinct ecoregions (Griffith et al. 2004, Zeug and Winemiller 2008, Vogl and Lopes 2009). Our study area consists of the lower ~⅓ of the watershed, covering an area of ~41,000 km^2 (Fig. 2.1). Previous work on the Brazos River watershed has identified substantial gradients in environmental conditions in the watershed that are the main drivers of nutrient and water quality conditions (Becker et al. *in revision*). The main-stem portion of the river in the study area is free of impoundments, but the river upstream and the major tributary sub-watersheds in the study area are regulated by dams (Zeug and Winemiller 2008). Land use across the entire lower Brazos watershed is predominantly agriculture and grazing (Zeug and Winemiller 2008, Becker et al. *in revision*); however, the individual sub-watersheds have distinct patterns of land use and environmental gradients (Becker et al. *in revision*). For this study, we sampled 33 sites across the lower Brazos watershed, which encompassed a combination of independent small tributaries in the Lower and Central watershed

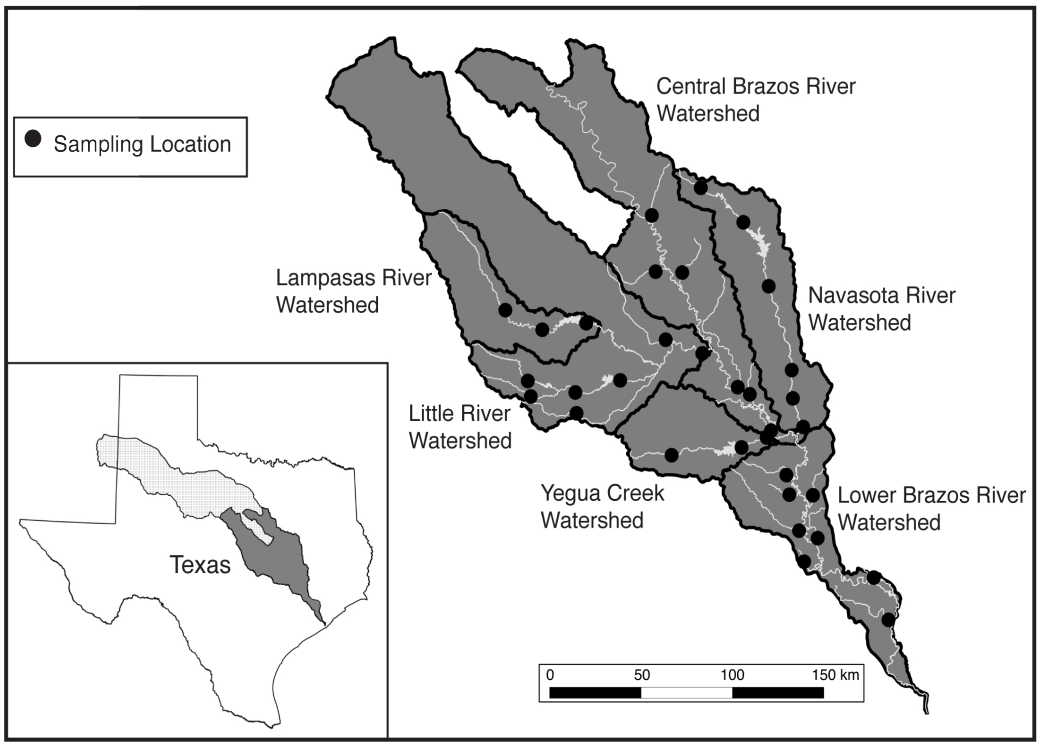

Fig. 2.1. Stream sampling locations and study catchments in the Brazos River watershed in Texas. Inset shows the entire Brazos River watershed. Light stippling indicates the upper Brazos watershed; grey area indicates focus of the present study. Dark lines represent watershed boundaries.

regions, as well as nested sites along the major tributaries, including the Navasota, Yegua, Little, and Lampasas Rivers (Fig. 2.1). Detailed site location information is presented in Becker et al. (*in revision*).

Stream Sampling and Laboratory Analyses

Physicochemical (habitat, environmental data, and aquatic nutrients), macroinvertebrate, and fish samples were collected concurrently from all sites for three field seasons of 2008-2009. Spring sampling occurred March – May 2008; summer sampling occurred June – August 2008; and winter sampling occurred November 2008 – January 2009. Detailed descriptions of sampling and processing procedures for nutrient, macroinvertebrate, and fish are in Becker et al. (*in revision*), Lash (2011), and Labay (2010), respectively and we provide a brief summary of sampling and processing procedures here. Site-specific physical

habitat characteristics were measured along multiple transects at each sampling location. Dominant substrate class (modified Wentworth scale), percent coverage of submerged vegetation (visual estimation), percent overhead canopy cover at mid-channel (spherical densiometer), and channel width were estimated at each transect where macroinvertebrates and fish were sampled. Average channel depth and stream velocity were also measured along the same transects using a Marsh-McBirney Flo-Mate 2000 electromagnetic flow meter (Hach Company, Fredrick, MD). For data analysis, the multiple transects were averaged for a site. Water temperature (°C), dissolved oxygen (mg/L), and pH were measured at each site with YSI™ sondes (Model 556 or Model 85, Yellow Springs, OH).

Water for nutrient analysis was collected in acid-washed 2-L brown Nalgene™ bottles. Bottles were rinsed 3x with site water prior to sample collection. Bottles were placed in coolers on ice until processed in the lab within 24 – 48 h of collection. In the lab, samples were immediately analyzed or divided into subsamples and preserved for later analysis. Water analyses included in this study are particulate phosphorous (PP), particulate N (PN), suspended particulate organic matter (SPOM), non-volatile suspended solids (NVSS), nitrate (NO_3^-), ammonium (NH_4^+), soluble reactive phosphate (SRP), and dissolved organic carbon (DOC). Analysis methods are detailed in Becker et al. (*in revision*). For data analysis, the two duplicate samples for each analyte from each site were averaged. These parameters were chosen for inclusion in this analysis because (1) they had the widest range of response to physiographic and land-use parameters (Becker et al. *in revision*), and (2) the nutrients in the Brazos River are predominantly in the dissolved form and previous studies indicated that total and dissolved nutrients responded similarly to the same physiographic and land-use predictors, thus inclusion of both total and dissolved nutrient fractions is redundant (Becker et al. *in revision*).

Benthic macroinvertebrates were collected using a combination of kick

net, dip net and Hess sampling. Samples were fixed in 70% ethanol in the field and processed in the lab. Macroinvertebrates were typically identified to genus using traditional keys (Thorp and Covich 2001, Merrit et al. 2008). Fish were collected using a combination of seine, gill net, backpack electrofisher, and boat mounted electrofisher. Fish were collected from riffle, run and pool habitats at each site. If possible, fish were identified to species in the field and released. Fish not identified in the field or used as voucher specimens were euthanized in tricane methanesulfonate (MS-222) and preserved in 10% formalin before transfer to 70% ethanol. For both macroinvertebrate and fish sampling, multiple geomorphic habitats were sampled at each location to maximize the number of species or taxa collected. Sampling effort in each geomorphic unit was proportional to the amount of habitat found at each site. The lists of macroinvertebrate taxa, fish species, and abbreviations for each group are found in Tables 2.S1 and 2.S2.

Geographic Data

Geographic information system (GIS) data for the study region was extracted using a combination of ArcInfo 9.3 (ESRI, Redlands, CA) and Quantum GIS 1.8.0 (QGIS; OSGeo, Beaverton, OR). Elevation for each sampling location was extracted using digital elevation models (DEM) from the 2009 National Elevation Dataset at a 1-arc second resolution (~30m), available on the USGS National Map Seamless Server. Stream network data was from the USGS National Hydrography Dataset (http://viewer.nationalmap.gov/viewer/nhd.html?p=nhd). Two distance matrices were created using the tools in QGIS. First, in order to evaluate the patterns of geographic autocorrelation between sites (Wilkinson and Edds 2001) a linear distance matrix was created using the Vector Analysis Distance Matrix tool. This tool uses the point location data to create a matrix of straight-line distances between all of the site pairs (in kilometers; km). Then, to evaluate the patterns of

autocorrelation due to stream channel connection (Magalhães et al. 2002), a matrix of river distance was created using the Shortest Path tool between all site pairs. This tool calculates the shortest path between site pairs along a vector layer (in river kilometers; rkm), which in our case was the USGS National Hydrography Dataset for the basin.

Data Analysis

Recent studies have encouraged the use of multiple analytical techniques to elucidate the patterns of community concordance, instead of relying on a singular technique (Gioria et al. 2011, Padial et al. 2012). Therefore, we utilized a variety of analytical approaches and methods to assess the relationships between site location, riverine connectedness, physicochemical conditions, and biotic community structure. We initially assessed overall concordance between the macroinvertebrate and fish community biodiversity and taxa/species richness (*S*) by calculating Shannon-Weiner index (*H'*) and Pielou's evenness index (*J'*) for each community at each site. Pearson correlation was then used to assess relationships between these metrics in the macroinvertebrate and fish communities. We secondarily conducted a more detailed assessment of concordance in species composition as well as the biotic relationship to physicochemical and spatial arrangement using three subsequent techniques: (1) Mantel tests, (2) Procrustes analysis, and (3) partial Mantel tests (Peres-Neto and Jackson 2001, Grenouillet et al. 2008, Gioria et al. 2011, Legendre and Legendre 2012).

For the Mantel (as well as the subsequent partial Mantel tests and Mantel correlograms, see below), five matrices of site-pair distance or dissimilarity were utilized: (1) Geographic coordinate distance, (2) river channel distance, (3) physicochemical conditions dissimilarity, (4) fish community dissimilarity, and (5) macroinvertebrate community dissimilarity. Both geographic and river

channel distances have been used to evaluate spatial autocorrelation between biological communities (Wilkinson and Edds 2001, Murphy and Davy-Bowker 2005, Grenouillet et al. 2008, Landeiro et al. 2011). Physicochemical data was $\log_{10}(x)$ or $\log_{10}(x+1)$ transformed (except for pH), *z*-score standardized, and a Euclidian dissimilarity matrix was computed on the normalized data. Singletons (species with only one individual counted) were removed from the macroinvertebrate and fish data, and Bray-Curtis dissimilarity matrices were computed on $\log_{10}(x+1)$ transformed data (Bray and Curtis 1957, Gioria et al. 2011, Padial et al. 2012). Procrustes analyses were run on the first two axes of ordinations on the spatial (principal coordinate analysis, PCoA), physicochemical (principal component analysis, PCA), and fish and macroinvertebrate (both with correspondence analysis, CA) datasets (Gioria et al. 2011, Legendre and Legendre 2012, Padial et al. 2012). We then ran partial Mantel tests to assess the interaction between the physicochemical, macroinvertebrate, fish, and spatial datasets after controlling for the influence of physicochemical or spatial data. Partial Mantel tests are a commonly used and straightforward way to control for the effect of a covariate matrix and better assess the strength of correlation between different datasets (Grenouillet et al. 2008, Padial et al. 2012).

Again, we elected to use a variety of techniques to assess biota-physicochemical relationships, as each of the techniques has advantages and drawbacks, and there is variability in how the techniques respond to the type and structure of the data (Gioria et al. 2011, Padial et al. 2012). The strength of a Mantel correlation is assessed by the test statistic, r_m, which is bounded between -1 and +1 (Legendre and Legendre 2012). Mantel tests are a robust and flexible method commonly used in studies of community concordance and test the similarities between sites based on distance matrices, however the reliance on distance/ resemblance matrices has recently been criticized (Heino 2010, Gioria et al. 2011).

Thus, we additionally utilized Procrustes analyses to assess concordance between communities (Peres-Neto and Jackson 2001). Procrustes analysis has a similar goal as Mantel tests, but instead of a distance matrix, Procrustes analysis uses the results from any two multivariate ordinations to assess concordance (Peres-Neto and Jackson 2001). The strength of a Procrustes correlation is assessed by the statistic m^2, which can be converted into a correlation coefficient $r_p = \sqrt{(1 - m^2)}$, where higher values indicate stronger concordance (Legendre and Legendre 2012). Procrustes analysis is a more powerful method of assessing concordance between communities, but the increased degree of concordance may result from the reduced dimensionality through the use of ordination axes (Gioria et al. 2011).

In order to assess the spatial autocorrelation among the physicochemical, macroinvertebrate, and fish community data, Mantel correlograms were computed with the aforementioned geographic (in km) and river channel distance (in rkm) matrices. Sites were divided into 5-6 distance classes using Sturge's rule (see Legendre and Legendre 2012 for details). Geographic distance was split into 5 categories, while river channel distance was separated into 6. The shape of the Mantel correlogram can inform about the nature of the autocorrelation patterns, with consistently positive correlations at the nearest distance classes and negative correlations at the largest distance classes indicating gradients or disruptive steps, while variable correlations indicate patchy distributions (Legendre and Fortin 1989, Grenouillet et al. 2008). Through the use of permutation tests, they can be used to estimate a "zone of influence" where the distance between sites is compared to the correlation strength and direction, with positively correlated sites being more similar, and negatively correlated sites being more dissimilar (Fortin and Dale 2005, Grenouillet et al. 2008). To assess the concordance patterns independent of the large-scale physiographic gradients within the Brazos watershed identified in Becker et al. (*in revision*), a second set of correlograms was computed on

macroinvertebrate, fish, and physicochemical distance matrices detrended against the *x* and *y* Cartesian site coordinates (in Lat-Long degrees [°]; Borcard et al. 2011, Legendre and Legendre 2012).

Finally, we used symmetric co-correspondence analysis (CoCA) to describe the overall patterns of community concordance in the Brazos watershed and then utilized predictive CoCA to assess the performance of one taxonomic group to predict the other (ter Braak and Schaffers 2004, Schaffers et al. 2008). For each of the multivariate analyses, tests were run on the annual data as well as the individual sampling periods to evaluate changes in concordance over the course of the year. For the annual analyses, mean values were used for physicochemical data, while total individuals collected over the study period were used for macroinvertebrate and fish data. Seasonal analyses were run on the values for each dataset at each site in the respective season. Predictive CoCA was run in both "directions" to assess the ability of macroinvertebrate communities to predict fish communities and vice-versa.

Permutation tests were run to assess the significance of all Mantel, partial Mantel, and Procrustes analyses (n = 9999). Mantel, partial Mantel, and Procrustes tests were conducted at $\alpha = 0.05$ with Bonferroni corrections for multiple testing. The Mantel correlogram α was corrected using the sequential Bonferroni technique, which is the default method in the 'vegan' statistical package. Predictive ability of the CoCA was assessed using a jackknife, "leave-one-out", cross-validation (ter Braak and Schaffers 2004). Cross-validatory fit is generally much lower than the "explained variation" of other techniques, but is not measuring the same strength of the relationship (Schaffers et al. 2008). Positive values of cross-validatory fit are an implicit validation of the model, as positive values indicate better prediction than by random chance (Schaffers et al. 2008). All univariate statistics were performed using the JMP 9.0 (SAS, Inc., Cary, North Carolina) platform. Multivariate statistics

were performed in the R statistical environment (R Foundation for Statistical Computing), using the 'vegan' (Oksanen et al. 2012) and 'cocorresp' (Simpson 2011) packages.

Results

Species richness (*S*) between macroinvertebrate and fish communities was not correlated ($p = 0.408$); however, there were significant correlations between the communities for both Shannon Diversity (*H'*, $r = 0.36$, $p = 0.0374$) and Pielou's Evenness (*J'*, $r = 0.43$, $p = 0.0137$). In terms of community concordance patterns, Mantel correlation coefficients (r_m) were significant for nearly all data pairings, except for the spring sampling macroinvertebrate-physicochemical datasets ($p = 0.0036$, Table 2.1) and the summer sampling macroinvertebrate-geographic distance datasets ($p = 0.0034$), which were both marginally non-significant. Mantel correlation coefficients ranged from a low of $r_m = 0.23$ (summer sampling macroinvertebrate-geographic distance) to a high of $r_m = 0.81$ (river channel distance-geographic distance). Seasonally, the correlation between the fish and macroinvertebrate community was strongest during the summer sampling ($r_m = 0.47$). On an annual basis the macroinvertebrates were most strongly correlated with physicochemical conditions ($r_m = 0.50$), although during the spring sampling the community was most correlated with river channel distance ($r_m = 0.39$). The variation in the fish communities were typically most strongly correlated with physicochemical conditions both throughout the year, and seasonally ($r_m = 0.46$, 0.42, 0.44). Procrustes analysis of these same sets of data indicated very similar results as the Mantel tests (Table 2.1).

Partial Mantel tests on the annual data indicated that the macroinvertebrate and fish communities were significantly concordant even when the effect of geographic distance, river channel distance, or physicochemical dissimilarity was

Table 2.1. Mantel and Procrustes correlations between macroinvertebrate and fish communities, physicochemical conditions, and distance between sampling locations. Fish = fish community matrix, Inverts = macroinvertebrate community matrix, Physicochemical = physicochemical conditions matrix, Geographic = geographic distance matrix, RKM = river distance matrix. Bold p = significant at Bonferroni corrected p-value (0.0033 for Mantel tests, 0.0055 for Procrustes analysis).

	Mantel		**Procrustes**	
Annual	r_m	p	r_p	p
Fish v. Geographic	0.479	**0.0001**	0.564	**0.0001**
Fish v. RKM	0.451	**0.0001**	0.601	**0.0001**
Fish v. Physicochemical	0.622	**0.0001**	0.696	**0.0001**
Inverts v. Geographic	0.336	**0.0001**	0.455	**0.0002**
Inverts v. RKM	0.465	**0.0001**	0.555	**0.0001**
Inverts v. Physicochemical	0.499	**0.0001**	0.609	**0.0001**
Fish v. Inverts	0.572	**0.0001**	0.860	**0.0001**
Physicochemical v. Geographic	0.471	**0.0001**	0.460	**0.0015**
Physicochemical v. RKM	0.492	**0.0001**	0.586	**0.0001**
RKM v. Geographic	0.809	**0.0001**	0.854	**0.0001**
Spring				
Fish v. Geographic	0.390	**0.0001**	0.523	**0.0001**
Fish v. RKM	0.381	**0.0001**	0.583	**0.0001**
Fish v. Physicochemical	0.457	**0.0001**	0.477	**0.0008**
Inverts v. Geographic	0.381	**0.0001**	0.551	**0.0001**
Inverts v. RKM	0.386	**0.0001**	0.631	**0.0001**
Inverts v. Physicochemical	0.275	0.0036	0.035	0.0401
Fish v. Inverts	0.367	**0.0001**	0.426	0.0327
Physicochemical v. Geographic	0.449	**0.0001**	0.617	**0.0001**
Physicochemical v. RKM	0.442	**0.0001**	0.535	**0.0002**
Summer				
Fish v. Geographic	0.420	**0.0001**	0.560	**0.0001**
Fish v. RKM	0.395	**0.0001**	0.604	**0.0001**
Fish v. Physicochemical	0.416	**0.0001**	0.486	**0.0006**
Inverts v. Geographic	0.229	0.0034	0.390	**0.0030**
Inverts v. RKM	0.297	**0.0008**	0.504	**0.0003**
Inverts v. Physicochemical	0.343	**0.0004**	0.379	0.0117
Fish v. Inverts	0.474	**0.0001**	0.836	**0.0001**
Physicochemical v. Geographic	0.356	**0.0001**	0.467	**0.0006**
Physicochemical v. RKM	0.334	**0.0002**	0.452	**0.0014**
Winter				
Fish v. Geographic	0.3517	**0.0002**	0.541	**0.0001**
Fish v. RKM	0.3457	**0.0002**	0.520	**0.0002**
Fish v. Physicochemical	0.4374	**0.0001**	0.454	**0.0022**
Inverts v. Geographic	0.2388	**0.0012**	0.598	**0.0001**
Inverts v. RKM	0.4000	**0.0001**	0.744	**0.0001**
Inverts v. Physicochemical	0.4593	**0.0001**	0.557	**0.0001**
Fish v. Inverts	0.3937	**0.0001**	0.557	**0.0004**
Physicochemical v. Geographic	0.4521	**0.0001**	0.431	**0.0033**
Physicochemical v. RKM	0.5357	**0.0001**	0.527	**0.0002**

accounted for (all $p = 0.0001$; Table 2.2). The fish community did not show spatial structure related to river channel distance when either the effect of geographic distance or physicochemical dissimilarity was used as the conditioning dataset ($p = 0.834$ and 0.065, respectively). Conversely, the insect community did not exhibit additional spatial structure related to geographic distance, when river channel distance or physicochemical dissimilarity was the conditioning dataset ($p = 0.8859$ and 0.0546, respectively). Physicochemical conditions were not related to either geographic distance or river channel distance when the other was used as the conditioning dataset ($p = 0.017$ and 0.008, respectively).

In the seasonal analyses, partial Mantel correlations had a much lower number of significant correlations (Table 2.2). In the spring sampling, there was a significant correlation between the macroinvertebrate and fish communities when the physicochemical data were used as a conditioning dataset ($p = 0.002$) but only marginally significant when geographic distance was the conditioning dataset ($p = 0.003$) and not significant when river channel distance was the conditioning dataset ($p = 0.005$). In the summer sampling, there was higher concordance between the macroinvertebrate and fish communities ($r_m = 0.39 – 0.43$, Table 2.2), and the partial correlations were significant with all three sets of conditioning data (all $p < 0.001$). In the winter sampling, macroinvertebrate and fish communities were significantly correlated when either distance measure was used as a conditioning matrix ($p < 0.002$ for both).

Mantel correlograms assessing the spatial correlation between sites for the physicochemical, macroinvertebrate, and fish community data indicated similar trends whether using geographic or river channel distance. Correlations between the distance classes for all sets of data were in approximately the same range ($r_m \approx -0.1 – 0.25$). For all groups, closest distance classes were significantly positively correlated, while the farthest distance class was significantly negatively correlated

Table 2.2. Partial Mantel correlationsbetween macroinvertebrate and fish communities, physico-chemical conditions, and distance between sampling locations. Matrix in parantheses is the conditioning matrix. Fish = fish community matrix, Inverts = macroinvertebrate community matrix, Physicochemical = physicochemical conditions matrix, Geographic = geographic distance matrix, RKM = river distance matrix. Bold p = significant at Bonferroni corrected p-value (0.0033).

Annual	r_m	p
Fish v. Inverts (Geographic)	0.497	**0.0001**
Fish v. Inverts (RKM)	0.459	**0.0001**
Fish v. Inverts (Physicochemical)	0.386	**0.0001**
Fish v. Geographic (RKM)	0.218	**0.0012**
Fish v. Geographic (Physicochemical)	0.269	**0.0006**
Fish v. RKM (Geographic)	0.123	0.0834
Fish v. RKM (Physicochemical)	0.213	0.0165
Fish v. Physicochemical (Geographic)	0.512	**0.0001**
Fish v. Physicochemical (RKM)	0.515	**0.0001**
Inverts v. Geographic (RKM)	-0.765	0.8859
Inverts v. Geographic (Physicochemica	0.132	0.0546
Inverts v. RKM (Geographic)	0.349	**0.0001**
Inverts v. RKM (Physicochemical)	0.291	**0.0013**
Inverts v. Physicochemical (Geographi	0.410	**0.0002**
Inverts v. Physicochemical (RKM)	0.351	**0.0004**
Physicochemical v. Geographic (RKM)	0.144	0.0166
Physicochemical v. RKM (Geographic)	0.021	0.008
Spring		
Fish v. Inverts (Geographic)	0.257	**0.003**
Fish v. Inverts (RKM)	0.258	0.0046
Fish v. Inverts (Physicochemical)	0.282	**0.0022**
Fish v. Geographic (RKM)	0.150	0.013
Fish v. Geographic (Physicochemical)	0.232	**0.0013**
Fish v. RKM (Geographic)	0.121	0.0634
Fish v. RKM (Physicochemical)	0.224	0.0099
Fish v. Physicochemical (Geographic)	0.343	**0.0003**
Fish v. Physicochemical (RKM)	0.348	**0.0001**
Inverts v. Geographic (RKM)	0.127	0.0339
Inverts v. Geographic (Physicochemica	0.300	**0.0001**
Inverts v. RKM (Geographic)	0.144	0.0476
Inverts v. RKM (Physicochemical)	0.307	**0.002**
Inverts v. Physicochemical (Geographi	0.126	0.1026
Inverts v. Physicochemical (RKM)	0.126	0.1088
Physicochemical v. Geographic (RKM)	0.174	0.0085
Physicochemical v. RKM (Geographic)	0.150	0.0448
Summer		
Fish v. Inverts (Geographic)	0.428	**0.0001**
Fish v. Inverts (RKM)	0.407	**0.0001**
Fish v. Inverts (Physicochemical)	0.388	**0.0001**
Fish v. Geographic (RKM)	0.185	0.0046
Fish v. Geographic (Physicochemical)	0.319	**0.0001**
Fish v. RKM (Geographic)	0.105	0.1222
Fish v. RKM (Physicochemical)	0.299	**0.0013**
Fish v. Physicochemical (Geographic)	0.315	**0.0007**
Fish v. Physicochemical (RKM)	0.329	**0.0005**
Inverts v. Geographic (RKM)	-0.020	0.6038
Inverts v. Geographic (Physicochemica	0.121	0.0636
Inverts v. RKM (Geographic)	0.195	0.0139
Inverts v. RKM (Physicochemical)	0.206	0.0168
Inverts v. Physicochemical (Geographi	0.288	**0.0022**
Inverts v. Physicochemical (RKM)	0.272	0.0039
Physicochemical v. Geographic (RKM)	0.156	0.0106
Physicochemical v. RKM (Geographic)	0.082	0.1638
Winter		
Fish v. Inverts (Geographic)	0.341	**0.0003**
Fish v. Inverts (RKM)	0.297	**0.0019**
Fish v. Inverts (Physicochemical)	0.241	0.0064
Fish v. Geographic (RKM)	0.131	0.027
Fish v. Geographic (Physicochemical)	0.192	0.0089
Fish v. RKM (Geographic)	0.111	0.099
Fish v. RKM (Physicochemical)	0.147	0.0551
Fish v. Physicochemical (Geographic)	0.333	**0.0001**
Fish v. Physicochemical (RKM)	0.318	**0.0001**
Inverts v. Geographic (RKM)	-0.157	0.9954
Inverts v. Geographic (Physicochemica	0.039	0.2933
Inverts v. RKM (Geographic)	0.362	**0.0001**
Inverts v. RKM (Physicochemical)	0.205	0.0119
Inverts v. Physicochemical (Geographi	0.406	**0.0001**
Inverts v. Physicochemical (RKM)	0.317	**0.0004**
Physicochemical v. Geographic (RKM)	0.038	0.2763
Physicochemical v. RKM (Geographic)	0.324	**0.0001**

(Figs. 2.2A, 2.2B). The zone of influence for all three sets of data was approximately 80 km for geographic distance (except macroinvertebrates which was approximately 50 km) and approximately 225 rkm for river channel distance. Spatially detrended correlograms indicated significant autocorrelation only in the fish community at the nearest distance class (Fig. 2.2C).

Symmetric CoCA indicated a modest concordance on an annual basis, with spring sampling having the highest concordance (λ_{1+2} = 0.09 and 0.44 in the first two axes, respectively; Table 2.3, Figure 2.3), and decreasing during subsequent samplings (Table 2.3). Much of the ordination on the first symmetric CoCA axis is influenced by relatively rare species in each taxonomic group occurring at the same few sites (Figure 2.3; see supplemental material for seasonal trends). Predictive CoCA indicated that the highest cross-validatory fit was in the annual data (5.7% and 4.7%, respectively). The seasonal trends were nearly reversed for the two analyses. The ability of the macroinvertebrate community to predict patterns in the fish community was very low in the spring sampling (0.7%) and rose during the year to 5.1% (Table 2.3). In the opposing scenario, where the fish community was used to predict the invertebrate community, the spring sampling cross-validatory fit was 3.1%, rose to 4.1% for summer, and dropped to 2.5% in winter.

Table 2.3. Co-correspondence Analysis (CoCA) Results. SymCoCA = symmetric CoCA, PredCoCA = predictive CoCA, λ = the percent fit in the first two SymCoCA axes, Fit = cross-validatory fit percent, Inverts = macroinvertebrate community, Fish = fish community (Arrow indicates the predictive direction), Axes = the number of axes in the model with the maximum cross-validatory fit.

	SymCoCA	**PredCoCA**			
	λ	Fit (Inverts -> Fish)	Axes	Fit (Fish -> Inverts)	Axes
Annual	9	5.73	5	4.71	2
Spring	44.3	0.70	2	3.10	4
Summer	26.3	2.38	3	4.08	1
Winter	17.9	5.06	7	2.53	1

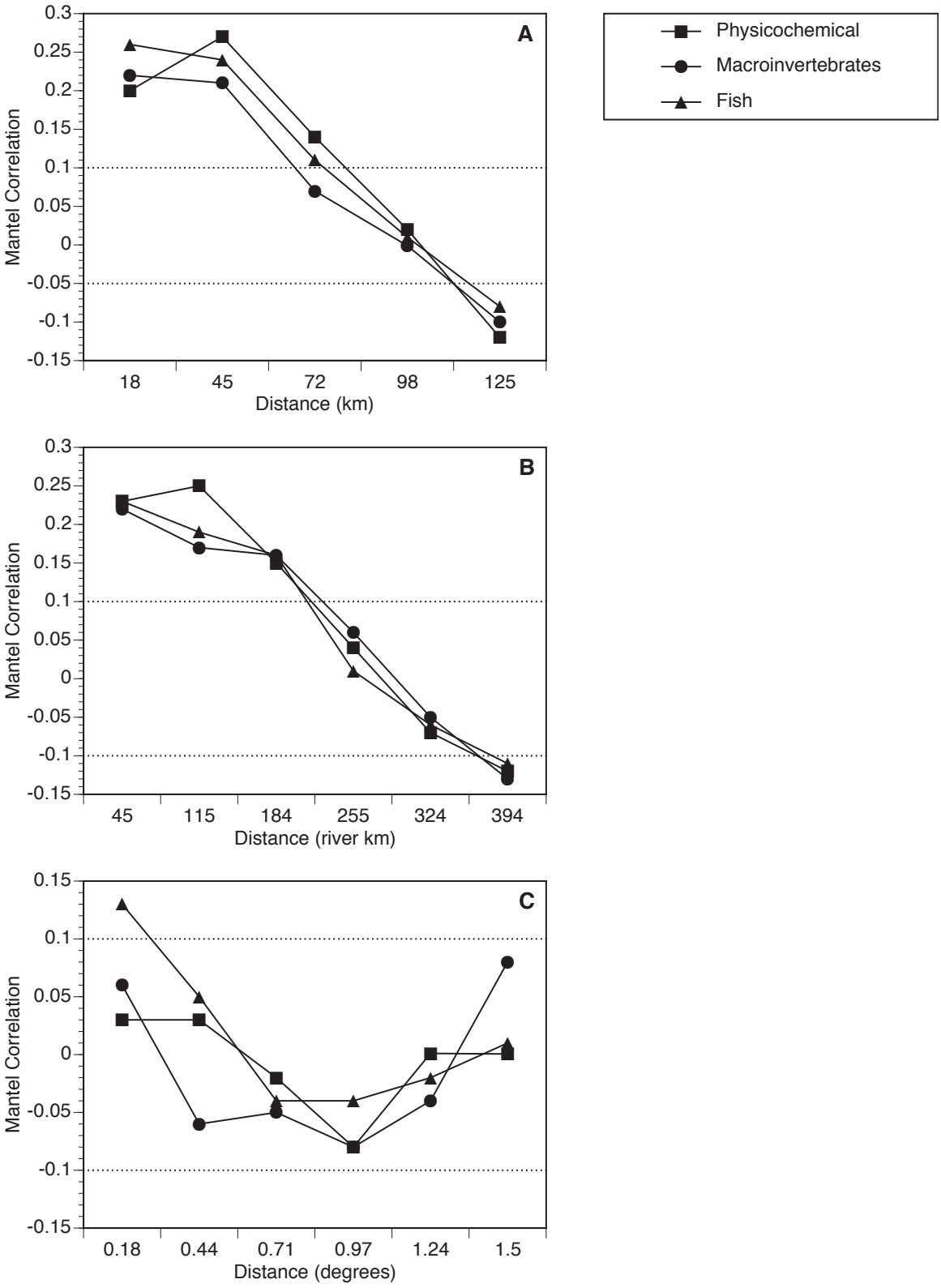

Fig. 2.2. Mantel correlograms for physicochemical, macroinvertebrate, and fish community data in the Brazos River watershed. A. Correlogram based on geographic distance between sites. B. Correlogram based on river channel distance betwee sites. C. Correlogram after detrending based on the Cartesian coordinates.

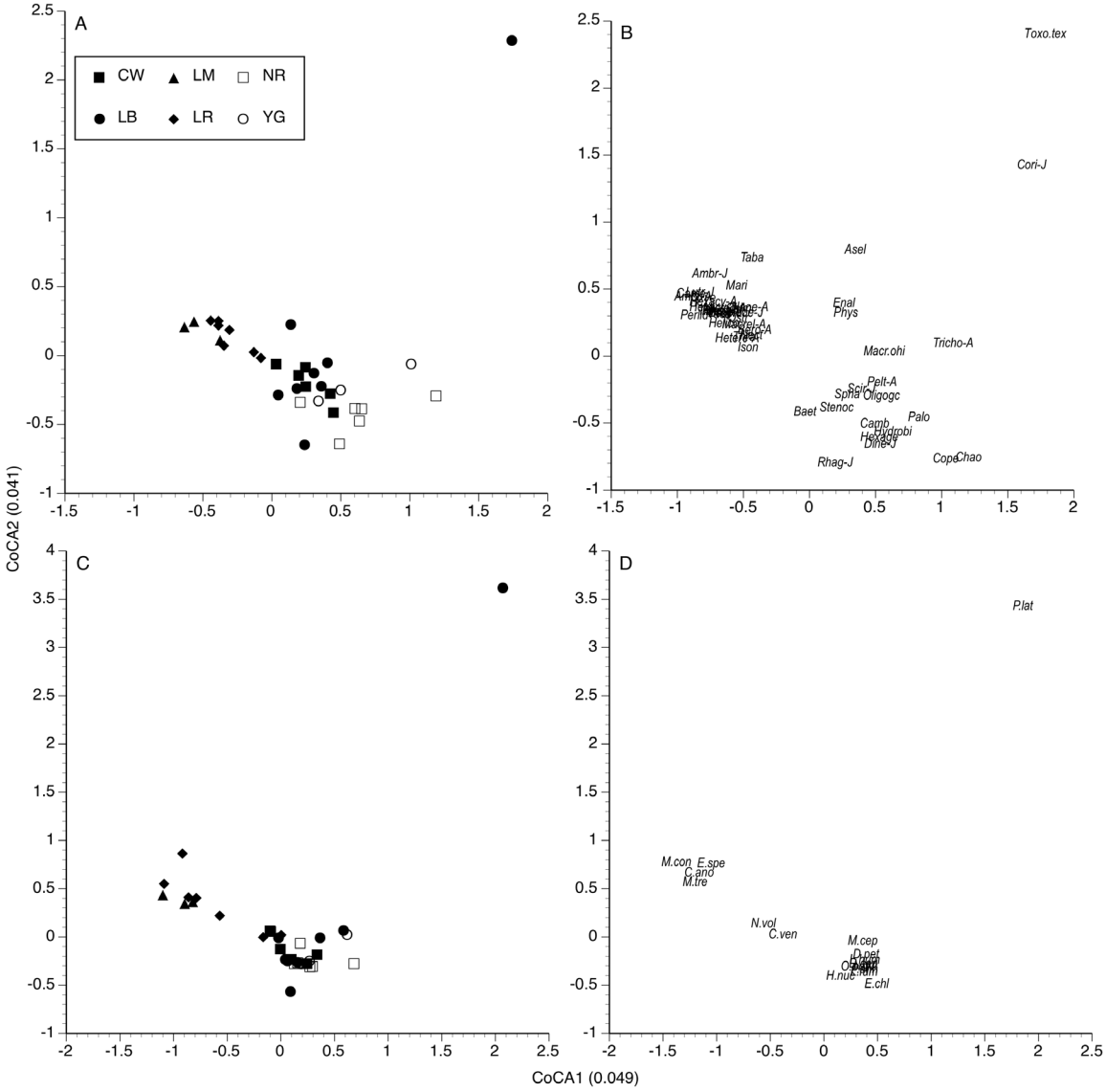

Fig. 2.3. Symmetric co-correspondence analysis (CoCA) of the annual data for macroinvertebrate and fish communities. CW = central Brazos River watershed, LB = Lower Brazos River watershed, LM = Lampasas River watershed, LR = Little River watershed, NR = Navasota River watershed, YG = Yegua Creek watershed. A. Site ordinations for macroinvertebrate community data. B. Species ordinations for macroinvertebrate community data. C. Site ordinations for fish community data. D. Species ordinations for the fish community data. Species shown have a greater than 25% fit, and have more than 50 individuals collected during the study. Species abbreviations are given in Tables S1 and S2.

Discussion

The amount of concordance between communities composed of diverse taxa and the degree to which community patterns are driven by external environmental influences or internal biotic interactions are core topics of community ecology (Currie 2007, Dray et al. 2012). This study highlights the degree to which the multiple measures of community concordance are inter-related and the difficulty in separating the various factors that can influence patterns of biodiversity and community concordance on a landscape scale (Fortin and Dale 2005). It also highlights the utility of using multiple taxa and environmental data, collected concurrently, as well as multiple analytical techniques to better elucidate landscape patterns (Paavola et al. 2006, Grenouillet et al. 2008, Gioria et al. 2011). Cross-taxa concordance patterns are notoriously variable and the statistical techniques and analysis scale used in evaluating these patterns can influence the interpretation (Paavola et al. 2006, Heino 2010, Johnson and Hering 2010). However, in the present study we were able to detect consistent community concordance patterns using multiple analyses. Consistent with our expectations, we found that there were patterns in concordance between the macroinvertebrate and fish communities, and that they were primarily influenced by common response to landscape scale gradients. However, the ability of one community to predict the other was relatively low. Thus, while there were concordant changes in the macroinvertebrate and fish communities of the Brazos River watershed, it is likely that they mostly represent common response to exogenous environmental factors and less represent endogenous trophic or competitive interactions.

The use of broad scale measures of diversity (*S*, *H'*, *J'*) indicated inconsistent patterns between macroinvertebrate and fish taxa. Species richness is one of the most commonly-used metrics of community structure (Heino 2010). Species richness has been used to assess concordance both between macroinvertebrates

and fish, as well as other aquatic taxa, and findings indicate that the strength of correlation is often weak but significant (Heino 2010). Similarly, Johnson and Hering (2010) recently included the same measures of richness, diversity and evenness as this study in an assessment of community concordance patterns in European streams and also found similarly variable results. The authors found that differences in concordance between the three community-wide metrics (S, H', and J') depended on the stream site's position in the landscape (i.e., highland verus lowland), indicating that the concordance, assessed broad metrics is largely context dependent (Johnson and Hering 2010). Broad measures of community structure such as S, H' and J' may have utility when assessing concordance within or between taxa with strong interactions, but the utility is likely limited between taxa with widely differing size ranges and ecology (Heino et al. 2005, Wolters et al. 2006, Padial et al. 2012). As suggested by Heino (2010) and Johnson and Hering (2010), our results confirm that it is important to use more detailed assessments of community concordance, especially if the goal is to generalize results to larger ecosystems or identify indicator species/taxa for use in conservation and management planning.

Concordance in Community Assemblage and Distance Measures

On an annual basis, physicochemical, macroinvertebrate, and fish data in the Brazos River watershed were all moderately but significantly concordant (Table 1). The strongest correlations were observed in the physicochemical-fish and macroinvertebrate-fish pairings. The PCoA, PCA, and CA ordinations of the sites showed that qualitatively similar groupings of sites in each of the physicochemical, macroinvertebrate, and fish datasets (Fig. 2.S1), and this result is consistent with the patterns found in Becker et al. (*in revision*), Labay (2010), and Lash (2011) on the analyses of the individual datasets. In these studies, consistent west-to-east,

largely upstream-downstream, gradients were found in the nutrient and community data. The groupings are consistent with ecoregion transitions, rainfall gradients, and longitudinal community development along the river network (Vannote et al. 1980, Grenouillet et al. 2008, Becker et al. *in revision*). For example there is a strong longitudinally influenced rainfall gradient in the watershed, with the drier, western portions of the watershed receiving ~79 cm of rainfall annually while the eastern portions of the watershed receive ~114 cm (Becker et al. *in revision*). Additionally, the two most distinct ecoregions, the Edwards Plateau and Western Gulf Coast Plains fall along this same gradient, while the Texas Blackland Prairie and East Central Texas Plains are centrally located, both geographically and in terms of the community compositions (Becker et al. *in revision*)

Seasonally, the overall strength of the correlations among the physicochemical, macroinvertebrate, and fish data in the Brazos River watershed were somewhat lower than the annual data (Table 2.2), but the same general patterns were still apparent. The fish-physicochemical and fish-macroinvertebrate pairings often had the higher correlation strengths, while weaker correlations were often with between the macroinvertebrates and physicochemical datasets. The lack of concordance between macroinvertebrates and physicochemical conditions in the spring sampling was likely driven by higher flows connecting and homogenizing otherwise distinct habitats (Thomaz et al. 2007). For example the average flow of the mainstem Brazos River during the spring sampling at the USGS gauging station nearest our most downstream site was ~4× the flow of the summer sampling and ~7× the flow of the winter sampling. The seasonal patterns of concordance found in the subtropical Brazos River appear to be more consistent than those in the tropical Paraná River, Brazil (Padial et al. 2012). Although our study had a similar r_m between macroinvertebrates and fish to the study of the Paraná River when the analyses were significant, the Paraná River study indicated much more variable patterns of

significance between samplings. In their two year study, encompassing both wet and dry seasons, only one of their fish-macroinvertebrate pairings was significant (Padial et al. 2012). When assessing the concordance between environmental and distance matrices with the macroinvertebrate and fish communities, the patterns in the Brazos River were more consistent as well as having typically stronger r_m (Padial et al. 2012).

The macroinvertebrate and fish communities appear to respond to both biotic interactions as well as spatial and environmental gradients. Unlike Padial et al. (2012), we did not find evidence that the macroinvertebrate and fish communities were primarily controlled by spatial effects and biological interactions. Instead our results indicated that environmental gradients were a significant force that both communities respond to. The partial Mantel tests indicated that when spatial or physicochemical patterns were controlled for, there were often still significant biotic correlations, however seasonally this was not universal (Table 2.2). Additionally, there were often spatial or physicochemical data that explained the majority of the distribution within the individual macroinvertebrate or fish datasets (Table 2.2). Finally, the spatially detrended Mantel correlograms indicated that only fish at the nearest distance class exhibited autocorreation. There has been recent discussion about the relative influence of environmental conditions on community composition (Bahn and McGill 2007, Currie 2007), however in the Brazos River watershed, multiple measures of concordance indicated that environmental (in our case, physicochemical) influences were important after accounting for spatial effects or biotic interactions.

Despite the relatively high correlation between the geographic and river channel distances, there were differences in how the physicochemical, macroinvertebrate, and fish communities responded to each distance matrix. To our knowledge only Landiero et al. (2011) has explicitly compared the influence

of geographic distance and river channel distance on macroinvertebrate and fish communities, or the implications of using one type of measure versus another.

In the Brazos River, physicochemical conditions, macroinvertebrate and fish communities were on average more strongly correlated with river channel distance than geographic distance. It has been suggested that river channel distance may be a more ecologically meaningful measure of distance between river locations, but geographic location can potentially preserve regional environmental and catchment features that are as important as the distance between sites on a river network (Wilkinson and Edds 2001, Magalhães et al. 2002, Murphy and Davy-Bowker 2005). Additionally, the "best" distance measure is likely to depend on the dispersal ability of the organism or community in question, which can change depending on the life stage of some organisms, such as many macroinvertebrates (Landeiro et al. 2011). The use of distance matrices in the Mantel, partial Mantel, and Procrustes analyses precluded a direct assessment of Cartesian location, thus geographic distance likely represents regional spatial structure, while river channel distance represents a better measure of network connection between sites (Magalhães et al. 2002, Murphy and Davy-Bowker 2005). Cartesian coordinates were used to account for large-scale spatial trends (Magalhães et al. 2002), as we utilized Cartesian coordinates as a spatial detrending tool for the second set of correlograms (Fortin and Dale 2005, Legendre and Legendre 2012). Additionally, they were incorporated into the analyses by Becker et al. (*in revision*), Labay (2010), and Lash (2011). Better understanding of the utility of each measure will take more direct comparison.

In Mantel and Procrustes analysis it appears river channel distance was a better measure than geographic distance, however interpretation of the partial Mantel tests is not clear-cut. There were differences in how macroinvertebrate and fish communities wee correlated with either river channel or geographic distance, both on an annual basis and seasonally. Thus, it appears that they do relate differently to

the community data, but the interpretation of those differences is unclear.

Spatial and Seasonal Interactions

On an annual basis, macroinvertebrates and fish communities in the Brazos River watershed were weakly, but significantly concordant no matter if the analysis was conditioned on geographic distance, river channel distance, or physiochemical data (Table 2.2). Additionally, both fish and macroinvertebrate communities were significantly correlated to physicochemical conditions when either measure of distance was used as the conditioning matrix. However, macroinvertebrate communities were not correlated with linear distance when either physicochemical or river channel distance was used as the conditioning matrix, indicating a stronger response to habitat physicochemical and stream network connections than to regional patterns (Grenouillet et al. 2008). In contrast, fish were more strongly correlated with geographic distance, indicating a greater influence of regional-level environmental control. Even in the spatially independent analyses, macroinvertebrates and fish were likely responding a combination local environmental and habitat conditions as well as through community interactions (Grenouillet et al. 2008, Johnson and Hering 2010, Dolph et al. 2011). In reality, separating the independent influences of physiochemical gradients, spatial autocorrelation, and community interactions will take much more work, as often they are not truly independent (Legendre et al. 2002, Bahn and McGill 2007, Currie 2007).

Few studies have addressed changes in concordance patterns on a temporal basis (Lloyd et al. 2005, Thomaz et al. 2007, Padial et al. 2012). Thus, this study is one of the first to assess seasonal dynamics in community concordance and we found that patterns of concordance between macroinvertebrates and fish in the Brazos River exhibited some seasonal changes. In contrast to Padial et al. (2012),

we found that the concordance strength among fish and invertebrate communities was higher in the drier summer months. Additionally, during the spring sampling, the relationship between macroinvertebrates and fish was not significant when the analysis was conditioned on river channel distance, indicating that river connection was more important in structuring the communities (Thomaz et al. 2007). During the winter sampling, the relationship was not significant when physicochemical data were used as the conditioning matrix, indicating a stronger influence of habitat and nutrient influences on the community concordance. The stronger concordance in the summer could have been driven by a reduction in connectivity between sites in the smaller streams (Thomaz et al. 2007, Padial et al. 2012), however these results are in conflict with or findings from the symmetric CoCA.

In the Brazos River watershed the "zone of influence" or range of positive spatial autocorrelation (*sensu* Legendre and Fortin 1989) for the physicochemical and fish data was approximately 80 km for geographic distance and for macroinvertebrates it was ~50 km. However, using river channel distance, the zone of influence was ~200 rkm for all three datasets. For the physicochemical and fish datasets, these zone of influence distances using the geographic distances were slightly greater than the ranges presented in other studies (20 – 40 km), although most of the other studies were conducted in much smaller watersheds (100 – 1500 km^2; Wilkinson and Edds 2001, Lloyd et al. 2005, Grenouillet et al. 2008). The zone of influence distance for macroinvertebrates in the Brazos River watershed were substantially greater than most estimates for macroinvertebrates reported in the literature, which most often fall in the 6 – 20 rkm river channel distance range (Magalhães et al. 2002, Lloyd et al. 2005, Grenouillet et al. 2008). Although the number of studies which have examined zone of influence in physicochemical conditions and organisms at relatively large watershed scales is limited, Murphy and Davy-Bowker (2005) assessed macroinvertebrate assemblage patterns

~5700 sites across England and Wales using geographic distances found a zone of influence of approximately 150 km. Wilkinson and Edds (2001) assessed the spatial autocorrelation patterns in fish communities across a multi-ecoregion area in central North America and found a zone of influence of approximately 44 km. The relative distances to which sites were spatially autocorrelated in the Brazos River indicated a significant effect of regional patterns (Fortin and Dale 2005). It is possible that the relatively large distances between our study sites in the Brazos River precludes detection of the finer scale patterns of autocorrelation that have been identified in macroinvertebrate communities, often attributed to in-stream factors such as substrate and riparian condition or dispersal ability (Lloyd et al. 2005, Grenouillet et al. 2008). There are both local and regional influences on community composition and interaction and the scale of a study, and the specific metrics used, will likely affect the degree to which each influence is seen (Thomaz et al. 2007, Dolph et al. 2011).

The shape of the correlograms for all three datasets indicated a step-like pattern in the correlations between the distance classes, with the shorter distance classes being positively correlated and the largest distance classes being negatively correlated and the slope of this relationship changing substantially as the distance classes increased (Legendre and Fortin 1989, Wilkinson and Edds 2001). More importantly, when the data was spatially detrended (Legendre and Legendre 2012), only fish communities exhibited any significant spatial autocorrelation and only at the nearest distance class (~45 rkm). These results indicate that the patterns of species occurrence and physicochemical conditions seen at the large scale of the Brazos River watershed are largely driven by spatially-structured regional environmental patterns, whether directly measured or not (Murphy and Davy-Bowker 2005, Borcard et al. 2011, Legendre and Legendre 2012). Although stream fragmentation and dispersal ability of fish were not assessed in this study, the

significant autocorrelation in the fish community after detrending is evidence that there is some large- scale autocorrelation caused by dispersal between sites in the Brazos River (Grenouillet et al. 2008).

Concordance of Species Assemblages

The symmetric CoCA conducted with the annual data largely agreed with the annual Mantel and Procrustes analyses. Additionally, it gave insight into the community composition changes that occurred across the Brazos River watershed (ter Braak and Schaffers 2004, Gioria et al. 2011). Although there were a large number of wide-spread and generalist taxa (as indicated by ordinations near the origin on the CoCA figures), we found relatively distinct communities between the upper and lower portions of the Brazos drainage, with several taxa being highly associated with sites in these portion of the watershed (Fig. 2.3). However, comparing the annual symmetric CoCA in this study to the canonical correspondence analysis (CCA) technique used in Labay (2010) and Lash (2011), which related the fish and macroinvertebrate communities to environmental and land use predictors, indicated a much stronger response in both communities to environmental conditions than to community structure of the other set of taxa. The amount of variation explained in the first two axes (analogous to a "percent fit") in the CCAs was 30.9% and 37.1% for macroinvertebrates and fish, whereas it was only 9.0% in the symmetric CoCA used in this study. Although the percent fit was substantially lower using symmetric CoCA, many of the same species-site associations found in Labay (2010) and Lash (2011) were still apparent. Studies in other systems have found an improved fit when using CoCA versus CCA, however this is likely due to the community combinations having a higher degree of structural dependency between communities rather than trophic interactions (ter Braak and Schaffers 2004, Mykrä et al. 2008, Cvetkovic et al. 2010).

Seasonally, there were substantial changes in the amount of variation explained in the symmetric CoCA. Similar site ordination patterns were found across seasons: typically there were one or two sites that indicated largely unique communities in both sets of taxa, with the rest of the sites showing a gradual turnover of species in an upstream-downstream gradient (See Supplemental Figs. 2.S2-2.S4). However, the percent fit of the seasonal CoCA models were much higher than the annual CoCA model, with a high of 44.3% in the spring and dropping through the year to 17.9% in the winter sampling. This is in contrast to the patterns seen with the Mantel and Procrustes correlations, where the strongest correlation occurred in the summer. Again, it can be expected that the wetter Spring season should have lower concordance due to high flow homogenizing habitats (Thomaz et al. 2007). However, given the low annual concordance, we speculate that the elevated seasonal concordance is likely the result of seasonal community and habitat availability changes happening simultaneously in the two communities, rather than large increases in trophic interaction strength (Johnson and Hering 2010, Padial et al. 2012).

Our final goal was to assess the ability of the structure in the macroinvertebrate community to predict the structure of the fish community, and vice versa. The identification of surrogate groups or taxa has the potential to simplify monitoring programs and allow researchers to focus studies where they have in-house knowledge while still retaining broad application (Dolph et al. 2011, Padial et al. 2012). For surrogate taxa to be useful, the correlation should be strong (> 0.7; Heino 2010), which we did find in some of the Procrustes analyses. However, the results were variable, with none of the Mantel tests on the biotic data $r_m > 0.6$, and the predictive CoCA fits all < 6%, indicating, at best, moderate concordance between the two groups of taxa. As direct and explicitly predictive tool, CoCA is likely a better method to assess concordance (Padial et al. 2012). However, the

cross-validatory fit of a CoCA is likely to be lower than the percentage of variation explained in a CCA (Schaffers et al. 2008) and the values in this study were typically low (< 6%). Annually, the macroinvertebrate community was a slightly better predictor of the fish community than vice versa, however the percent fit was low in either case. Seasonally, the cross-validatory fit was variable, with the lowest value occurring in the spring, at a time when the percent fit of the symmetric CoCA was at the highest level. The ability of the macroinvertebrate community to predict the fish community was highest in the winter, indicating that they may be a larger food source during times of low primary productivity. The top-down effects of fish on the macroinvertebrate community were highest in the summer sampling, where there appears to be a stronger gradient from clear water sites of the Edwards Plateau to the more eutrophic sites downstream in the watershed (Becker et al. *in revision*). One likely explanation for the low cross-validatory fit between macroinvertebrates and fish is that concordance patterns are largely the result of similar habitat preferences (Johnson and Hering 2010). Certainly, there are trophic interactions between the two groups, but both directly use other groups, such as macrophytes as habitat (Johnson and Hering 2010). It has also been suggested that concordance will be stronger between groups of similarly sized organisms (Heino 2010), although results assessing this hypothesis are variable (Grenouillet et al. 2008). Our study provides evidence that care should be taken in selecting surrogate species or groups for monitoring programs in riverine systems.

Conclusions

Biotic communities across large complex areas likely respond to a combination of both the proximate physicochemical conditions as well as regional environmental gradients. Additionally, there are direct community and species-species interactions that will influence the abundance and distribution of species

present at a given site. Currently, there are no statistical methods that can directly parse exogenous from endogenous influences on community structure, thus researchers need to use an array of techniques to infer the cause of observed patterns (Fortin and Dale 2005, Grenouillet et al. 2008, Gioria et al. 2011). At the scale investigated here in the Brazos River, exogenous environmental gradients were likely the strongest influence on the makeup of macroinvertebrates and fish communities. All of the groups of data assessed in this study exhibited similar patterns of spatial autocorrelation, and when spatial detrending techniques were used, only the fish community showed any spatial autocorrelation, and only at the nearest distance class. Although other studies have detected significant spatial autocorrelation in macroinvertebrate communities at relatively small distances (< 20 rkm), the sites sampled in the present study were largely outside of the zone of influence identified by other authors (Lloyd et al. 2005, Grenouillet et al. 2008). Thus, given the spatial extent of our study, we were more likely to identify larger scale physiographic environmental effects that had been independently identified in related studies on the nutrients, macroinvertebrates, and fish of the Brazos River (Infante et al. 2009, Labay 2010, Lash 2011, Becker et al. *in revision*). Concordance can occur at both small and large scales, although the underlying causes are likely different at varying scales and for different taxa (Dolph et al. 2011, Padial et al. 2012). As with other recent work, we found relatively weak and variable predictive ability between the macroinvertebrate and fish taxa. This is further evidence that the use of surrogate groups to assess patterns of biodiversity or ecosystem health should be done with caution. Disentangling the role external environmental conditions and internal community interactions have in determining broad scale species distributions across taxa is critical to advancing the field of community ecology (Currie 2007), and doing so will improve our ability to understand species distributions and the potential impact of future species introductions and

extinctions.

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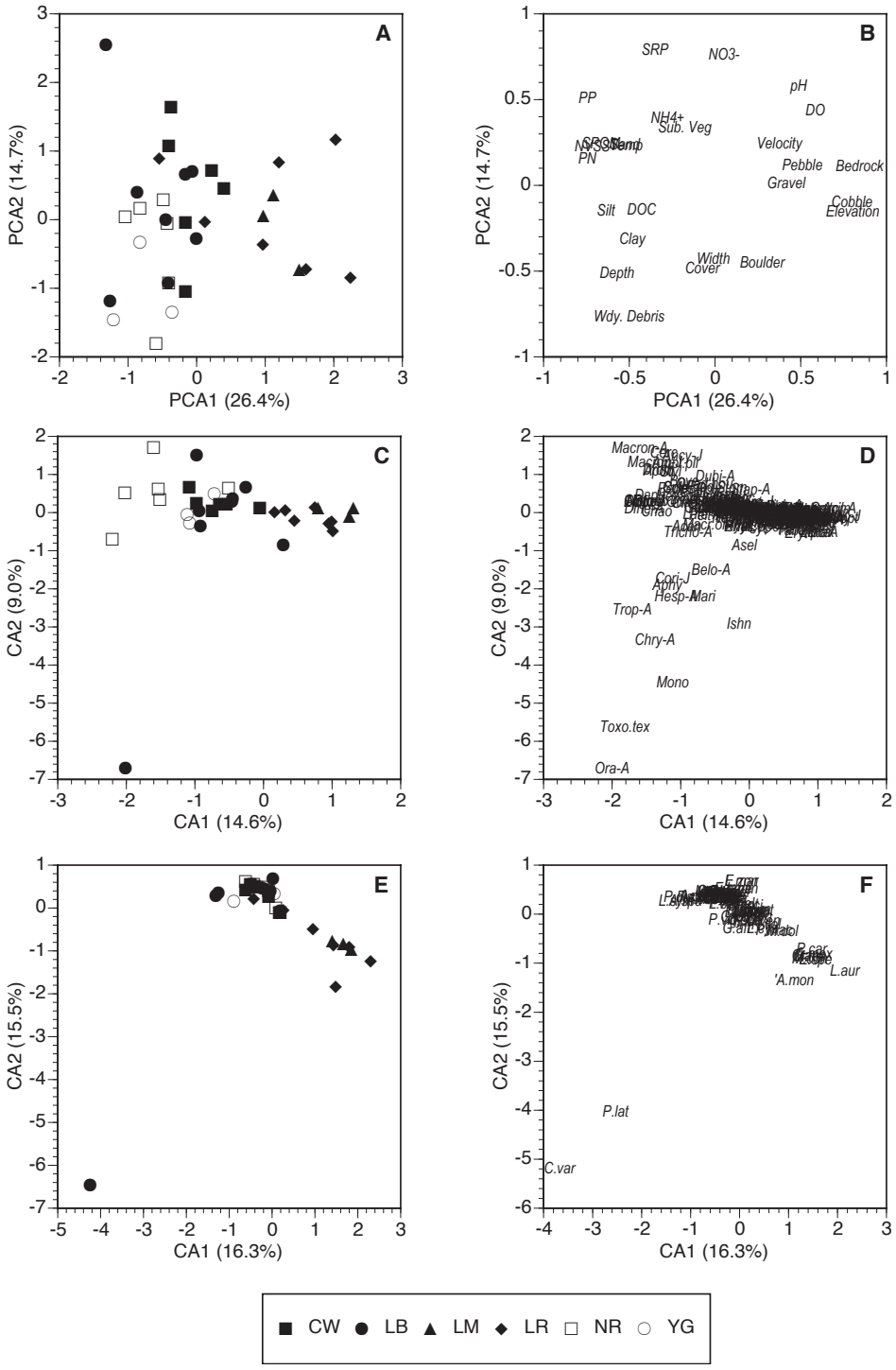

Fig. 2.S1. Ordinations on the annual data for physicochemical data (PCA), macroinvertebrate community (CA), and fish community (CA). A. Site ordinations for the physicochemical data. B. Physicochemical component ordinations for the present study. C. Site ordinations for the macroinvertebrate community data. D. Species ordinations for the macroinvertebrate community data. E. Site ordinations for the fish community data. F. Species ordinations for the fish community data. Species abbreviations are given in Tables 2.S1 and 2.S2. Please note that the scales may not be the same between ordinations.

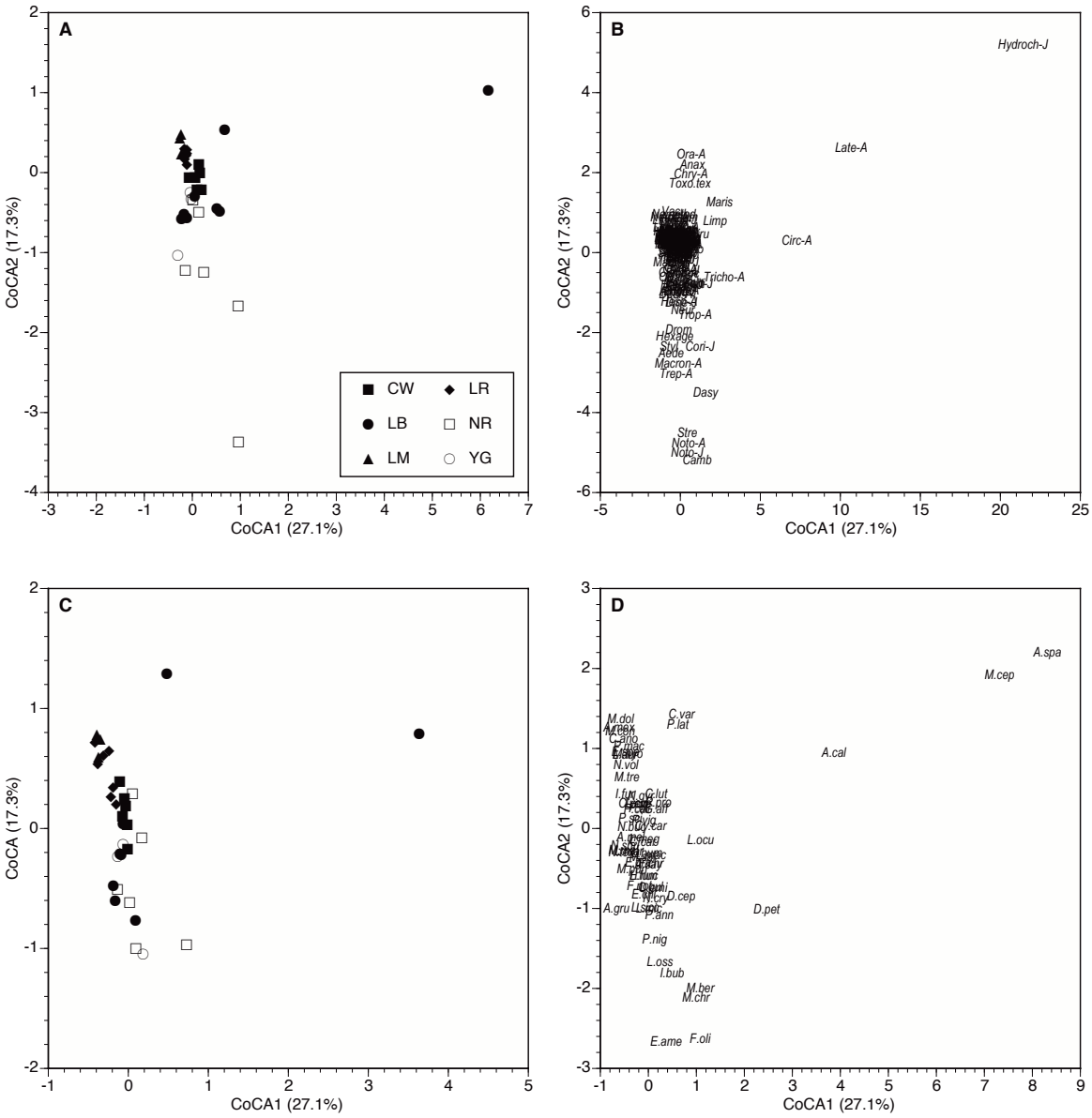

Fig. 2.S2. CoCA ordinations on the spring sampling for macroinvertebrate and fish communities in the Bra2.zos River watershed. A. Site ordinations for the macroinvertebrate community data. B. Species ordinations for the macroinvertebrate community data. C. Site ordinations for the fish community data. D. Species ordinations for the fish community data. Species abbreviations are given in Tables 2.S1 and 2.S2. Please note that the scales may not be the same between ordinations. Species ordination points have occasionally been altered slightly to improve readability. Changes do not alter the interpretation of the figures.

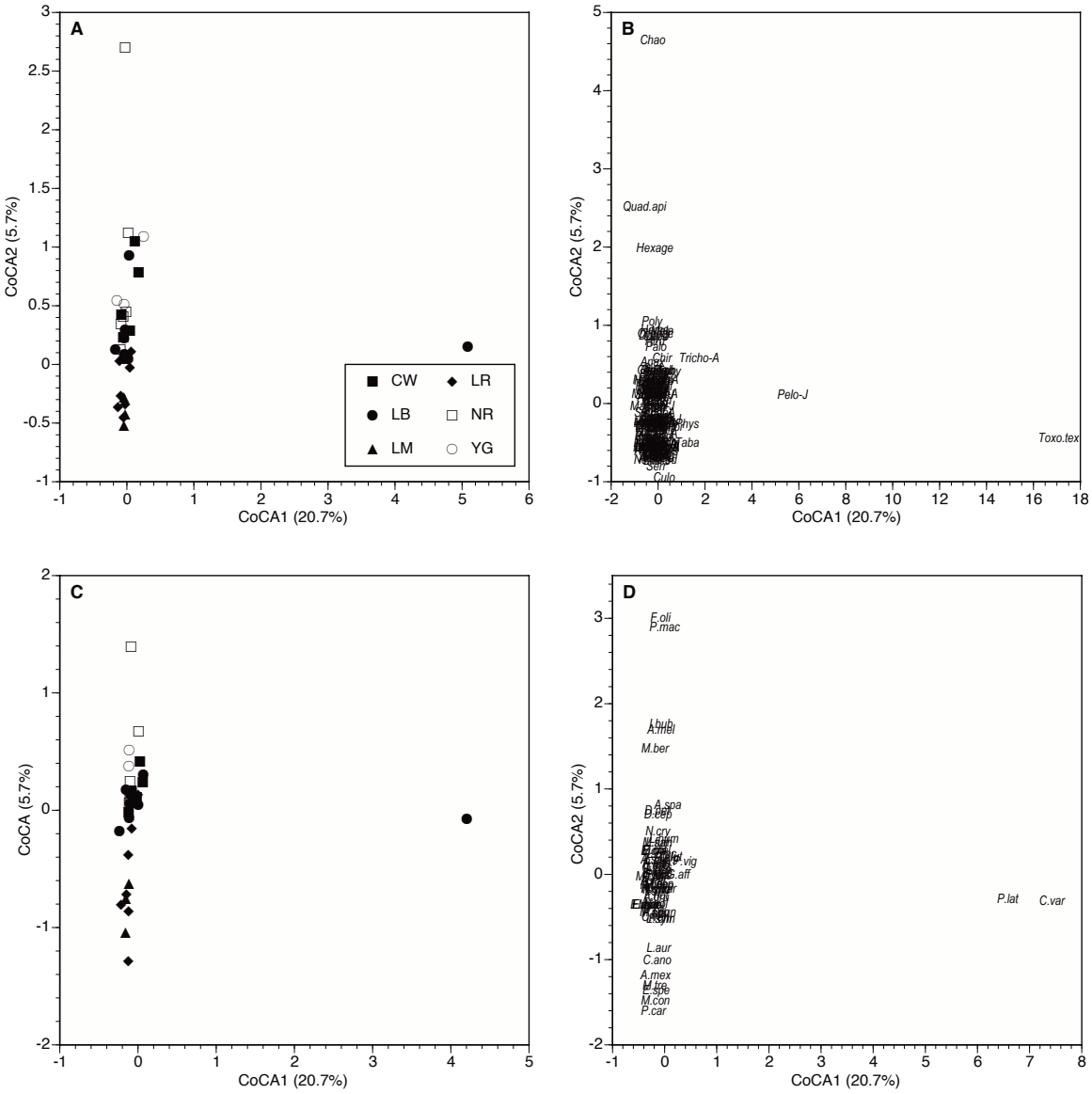

Fig. 2.S3. CoCA ordinations on the summer sampling for macroinvertebrate and fish communities in the Brazos River watershed. A. Site ordinations for the macroinvertebrate community data. B. Species ordinations for the macroinvertebrate community data. C. Site ordinations for the fish community data. D. Species ordinations for the fish community data. Species abbreviations are given in Tables 2.S1 and 2.S2. Please note that the scales may not be the same between ordinations. Species ordination points have occasionally been altered slightly to improve readability. Changes do not alter the interpretation of the figures.

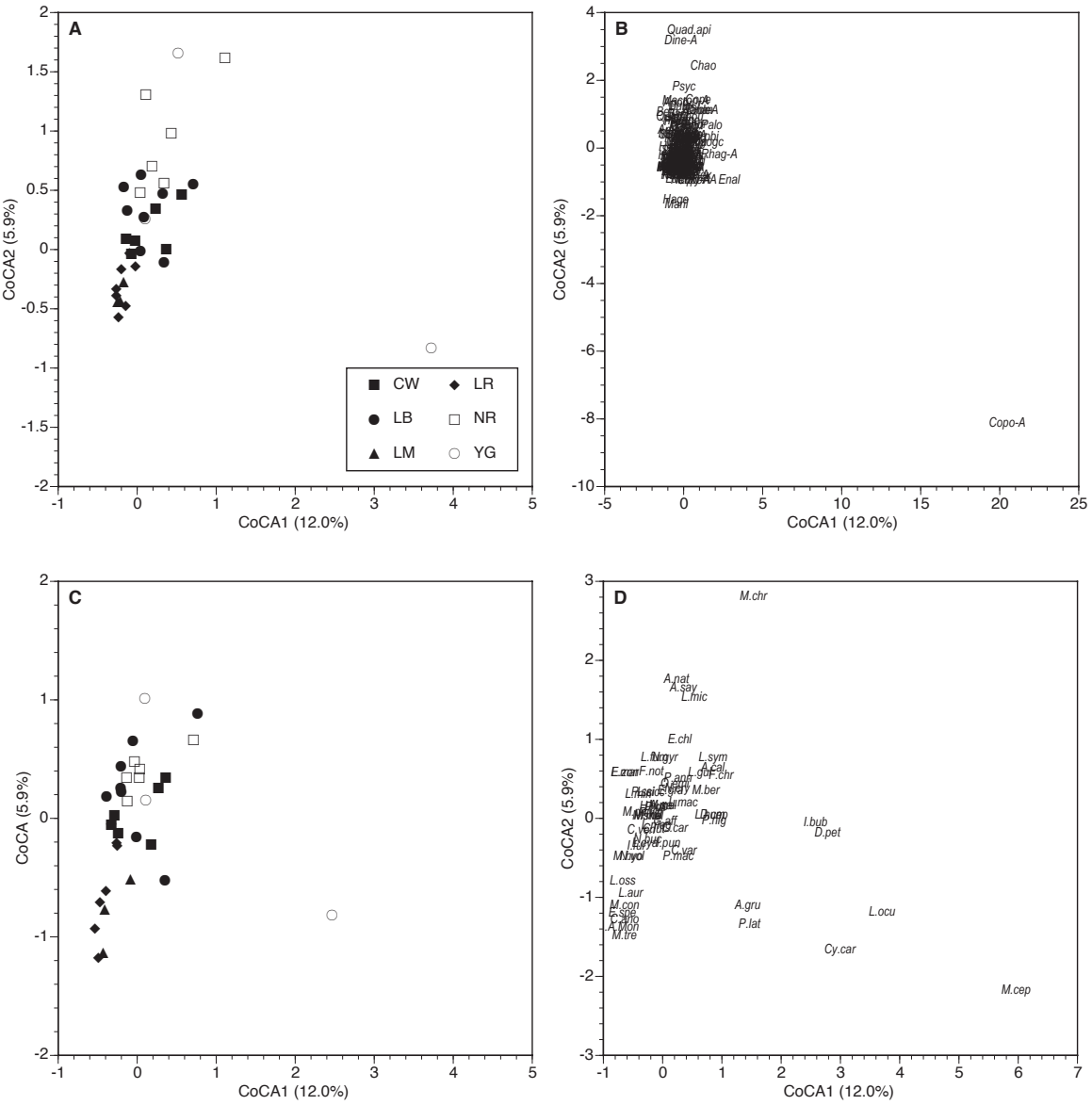

Fig. 2.S4. CoCA ordinations on the winter sampling for macroinvertebrate and fish communities in the Brazos River watershed. A. Site ordinations for the macroinvertebrate community data. B. Species ordinations for the macroinvertebrate community data. C. Site ordinations for the fish community data. D. Species ordinations for the fish community data. Species abbreviations are given in Tables 2.S1 and 2.S2. Please note that the scales may not be the same between ordinations. Species ordination points have occasionally been altered slightly to improve readability. Changes do not alter the interpretation of the figures.

Table 2.S1. Macroinvertebrates taxa found in the Brazos River watershed and abbreviations used in the present sttudy. -A or -J signifies adult or juvinile life stage.

Taxa	Abbreviation
Acanthagrion	Acan
Acarina	Acar
Acilius-A	Acil-A
Aedes	Aede
Amblema plicata	Ambl.pli
Ambrysus-A	Ambr-A
Ambrysus-J	Ambr-J
Anax	Anax
Ancyronx-J	Ancy-J
Aphylla	Aphy
Apobaetis	Apob
Argia	Argi
Aselus	Asel
Atherix	Athe
Attanueria	Atta
Baetis	Baet
Belostoma-A	Belo-A
Berosus-A	Bero-A
Berosus-J	Bero-J
Bezzia	Bezz
Boyeria	Boye
Brechmorhoga	Brec
Caenis	Caen
Calliobaetis	Call
Cambainae	Camb
Camelobaetis	Came
Carabidae-A	Cara-A
Centoptilum	Cent
Ceratopogon	Cera
Cercobrachys	Cerc
Chaoborus	Chao
Cheumatopsyche	Cheu
Chimarra	Chim
Chironominae	Chir
Chrysomelidae-A	Chry-A
Circulionidae-A	Circ-A
Collomebola	Coll
Copepoda	Cope
Copotomus-A	Copo-A
Corbicula	Corb
Corixidae-J	Cori-J
Corydalus	Cory
Crangonyx	Cran
Culex	Cule
Culicoides	Culi
Culoptila	Culo
Cymbiodyta-A	Cymb-A
Daphnia	Daph
Dasyhelea	Dasy
Dineutus-A	Dine-A
Dineutus-J	Dine-J
Dromogomphus	Drom
Dubiraphia-A	Dubi-A
Dubiraphia-J	Dubi-J
Eclichadidae-A	Ecli-A
Enallagma	Enal
Enochorus-J	Enoc-J
Ephyridae	Ephy
Epitheca	Epit
Erpetogomphus	Erpe
Erythemis	Eryt
Euporyphus	Eupo
Fallceon	Fall
Forcipomyia	Forc
Gammarus	Gamm
Gompuhs	Gomp
Hagenius	Hage
Haplius-J	Hapl-J
Helichus-A	Helich-A
Helicopsyche	Helico
Hemerodroma	Heme
Hesperocorixa-A	Hesp-A
Hetaerina	Heta
Heterelmis-A	Hetere-A
Heterelmis-J	Hetere-J
Heterocleon	Hete
Heterostronata-A	Hetero-A
Heterostronata-J	Hetero-J
Hexacylloepus-A	Hexacy-A

Table 2.S1 continued

Hexacylloepus-J	Hexacy-J	Nematoda	Nematom
Hexagenia	Hexage	Nematomorpha	Nematod
Hirudinae	Hiru	Nemotelus	Nemo
Hyallela	Hyal	Neochoroterpes	Neoc
Hydrobiidae	Hydrobi	Neoelmis-A	Neoe-A
Hydrochus-J	Hydroch-J	Neoelmis-J	Neoe-J
Hydroperla	Hydrosp	Neoperla	Neoper
Hydroptila	Hydropt	Neoporus-A	Neopor-A
Hydrospyche	Hydrope	Neoporus-J	Neopor-J
Ishnura	Ishn	Neurelipsis	Neur
Isonychea	Ison	Notonecta-A	Noto-A
Ithytrichia	Ithy	Notonecta-J	Noto-J
Laccophillus-A	Lacc-A	Nyctiophlax	Nyct
Laccophillus-J	Lacc-J	Nymphulella	Nymp
Lampsilis teres	Lamp.ter	Ocetis	Ocet
Lateralus-A	Late-A	Oligogchaeta	Oligogc
Leucotrichia	Leuc	Oligogomphus	Oligogo
Libellula	Libe	Ora-A	Ora-A
Limniporous-A	Limnoc-A	Orthocladinae	Orth
Limnocoris-A	Limnip-A	Ostracoda	Ostr
Limnocoris-J	Limnoc-J	Oxyethira	Oxye
Limpet	Limp	Palomonetes	Palo
Lipogomphus-A	Lipo-A	Pelocoris-A	Pelo-A
Lutrochus-A	Lutr-A	Pelocoris-J	Pelo-J
Lutrochus-J	Lutr-J	Peltodytes-A	Pelt-A
Maccaffertium	Macc	Peltodytes-J	Pelt-J
Macrelmis-A	Macron-A	Perlesta	Perlid
Macrelmis-J	Macron-J	Perlidae-J	Perles
Macrobrachium ohione	Macr.ohi	Petrophila	Petr
Macronychus-A	Macrel-A	Physidae	Phys
Macronychus-J	Macrel-J	Planaria	Planar
Marcromia	Marc	Planorbidae	Planor
Marilia	Maril	Plaudeus	Plau
Marisa	Maris	Plueroceridae	Plue
Mayatrichia	Maya	Polycentropus	Poly
Melanoides	Mela	Probezzia	Prob
Metrichia	Metr	Progomphus	Prog
Microcylleopus-A	Microc-A	Psephenus-J	Psep-J
Microcylleopus-J	Microc-J	Psychoda	Psyc
Microvelia-A	Microv-A	Quadrula apiculata	Quad.api
Microvelia-J	Microv-J	Quadrula houstonensis	Quad.hou
Monohelea	Mono	Ranatra-A	Rana-A
Nectopysche	Nect	Rhagovelia-A	Rhag-A

Table 2.S1 continued

Rhagovelia-J	Rhag-J
Sciomyzidae	Scio
Scirtes-J	Scir-J
Scrimidae	Scri
Serromyia	Serr
Sialis	Sial
Simulidae	Simu
Sphaeridae	Spha
Staphylinidae-A	Stap-A
Stenelmis-A	Stenel-A
Stenelmis-J	Stenel-J
Stenocron	Stenon
Stenonema	Stenoc
Stomatochloro	Stom
Stratiomys	Stra
streptocephalus	Stre
Stylurus	Styl
Tabanus	Taba
Tanypodinae	Tany
Telebasis	Tele
Tetragoneuria	Tetr
Thraulodes	Thra
Tipulidae	Tipu
Toxolasma texasesis	Toxo.tex
Trainodes	Trai
Travarella	Trav
Trepobates-A	Trep-A
Trichocorixa-A	Tricho-A
Tricorythodes	Tricor
Tritogonia verrucosa	Trit.ver
Tropisternus-A	Trop-A
Tropisternus-J	Trop-J
Vacupernis	Vacu

Table 2.S2. Fish species found in the Brazos River watershed and abbreviations used in the present study.

Genus	species	Abbreviation
Agonostomus	monticola	A.mon
Ameiurus	melas	A.mel
Ameiurus	natalis	A.nat
Amia	calva	A.cal
Aphredoderus	sayanus	A.say
Aplodinotus	grunniens	A.gru
Astyanax	mexicanus	A.mex
Atractosteus	spatula	A.spa
Campostoma	anomalum	C.ano
Carpiodes	carpio	C.car
Cyprinella	lutrensis	C.lut
Cyprinella	venusta	C.ven
Cyprinodon	variegatus	C.var
Cyprinus	carpio	Cy.car
Dorosoma	cepedianum	D.cep
Dorosoma	petenense	D.pet
Elassoma	zonatum	E.zon
Esox	americanus	E.ame
Etheostoma	chlorosoma	E.chl
Etheostoma	gracile	E.gra
Etheostoma	parvipinne	E.par
Etheostoma	spectabile	E.spe
Fundulus	chrysotus	F.chr
Fundulus	notatus	F.not
Fundulus	olivaceus	F.oli
Gambusia	affinis	G.aff
Hybognathus	nuchalis	H.nuc
Ictalurus	punctatus	I.pun
Ictalurus	furcatus	I.fur
Ictiobus	bubalus	I.bub
Labidesthes	sicculus	L.sic
Lepisosteus	oculatus	L.ocu
Lepisosteus	osseus	L.oss
Lepomis	auritus	L.aur
Lepomis	cyanellus	L.cya
Lepomis	gulosus	L.gul
Lepomis	humilis	L.hum
Lepomis	macrochirus	L.mac
Lepomis	marginatus	L.mar
Lepomis	megalotis	L.meg
Lepomis	microlophus	L.mic
Lepomis	miniatus	L.min
Lepomis	symmetricus	L.sym
Lythrurus	fumeus	L.fum
Macrhybopsis	hyostoma	M.hyo
Macrhybopsis	storeriana	M.sto
Membras	martinica	M.mar
Menidia	beryllina	M.ber
Micropterus	dolomieu	M.dol
Micropterus	punctulatus	M.pun
Micropterus	salmoides	M.sal
Micropterus	treculii	M.tre
Minytrema	melanops	M.mel
Morone	chrysops	M.chr
Moxostoma	congestum	M.con
Mugil	cephalus	M.cep
Notemigonus	crysoleucas	N.cry
Notropis	buchanani	N.buc
Notropis	shumardi	N.shu
Notropis	texanus	N.tex
Notropis	volucellus	N.vol
Noturus	gyrinus	N.gyr
Opsopoeodus	emiliae	O.emi
Percina	carbonaria	P.car
Percina	macrolepida	P.mac
Percina	sciera	P.sci
Pimephales	promelas	P.pro
Pimephales	vigilax	P.vig
Poecilia	latipinna	P.lat
Pomoxis	annularis	P.ann
Pomoxis	nigromaculatus	P.nig
Pterygoplichthys	disjunctivus	P.dis
Pylodictis	olivaris	P.oli

CHAPTER III

MICROBIAL FUNCTION AND BIOGEOGRAPHY IN A LARGE, COMPLEX RIVERSCAPE

Abstract

We studied the large-scale patterns of bacterial metabolic function and abundance in relation to nutrient concentrations in a large sub-tropical river. We found that unlike many systems in more temperate regions, bacterial production and growth efficiency were not related. Likely, the metabolic maintenance costs of bacteria are higher and more variable in this sub-tropical region. Additionally, both cell growth and maintenance are highly influenced by the amount of labile organic carbon sources, presumably from in-stream production. The link between bacterial community composition and function in the Brazos River watershed was weak. Bacterial production was related to total community abundance, to a point, however sites with extremely high bacterial abundances did not have resulting high production rates, and none of the investigated bacterial groups were correlated with increases in bacterial production. Bacterial communities have a large amount of functional redundancy, so at a coarse level of assessment, it is not surprising that these relationships would be weak. Finally, there were shifts in the overall community composition in relation to nutrients and basin position of the sampling site. While a portion of this was driven by bacterial abundance, there were differences between how some bacteria responded to nutrient differences in the watershed. Most bacteria in the Brazos River watershed were correlated with particulate loading, β-proteobacteria were highest in areas with elevated NO_3^- concentrations and *Actinobacteria* were highest in areas with elevated SRP. While

there were differences between β-proteobacteria and *Actinobacteria*, we did not find evidence for stronger competitive or exclusionary interactions between the groups.

Introduction

Bacterial communities constitute critically important, but frequently under-sampled component of all aquatic ecosystems. Bacteria are one of the most abundant and diverse forms of life on the planet (Whitman et al. 1998) and are involved in and essential to nearly every biogeochemical cycle (Prosser et al. 2007, Falkowski et al. 2008). Bacteria mediate processes that facilitate the use of carbon (C), nitrogen (N), and phosphorus (P) by higher organisms, and although the vast majority of bacterial groups are heterotrophic, bacteria communities exert a great deal of influence on "bottom-up" controls of ecosystem function and biogeochemical cycling (Cotner and Biddanda 2002, Falkowski et al. 2008). On a global scale bacteria represent a C pool of 60 - 100% of the amount contained in all plants (Whitman et al. 1998), and serve as a critically important food resource for many organisms that rely on the decomposition of organic matter (Meyer 1994). Heterotrophic bacteria are also responsible for processing large amounts of non-living organic (detrital) C and nutrients into forms that can be used by higher trophic level organisms (Maranger et al. 2005, Falkowski et al. 2008). From an ecosystem perspective, it is estimated that bacteria control transformation and use of approximately half of the organic C input to rivers (Cole et al. 2007). This processing by pelagic-, sedimentary-, and biofilm-bacterial communities is one of the major contributors to the approximately 0.75 Pg of carbon that is off-gassed as CO_2 in inland water ecosystems (Cole et al. 2007). Despite the clear importance of bacteria to food web interactions and ecosystem processes (Hall and Meyer 1998), relatively little is known about the interactions between the abundance and composition of bacterial communities, environmental physicochemical conditions,

and bacterial metabolic function, and in aquatic systems and riverine ecosystems in particular (Rubin and Leff 2007, Ochs et al. 2010).

In freshwater ecosystems one of the major functions that bacterial communities mediate is the processing of organic matter (OM; Cotner and Biddanda 2002, Battin et al. 2008). Much of the OM in freshwater systems is derived and delivered from terrestrial systems, and there is typically a downstream longitudinal gradient in the relative importance of allochthonous (OM from terrestrial sources) versus autochthonous (OM from riverine sources) production (Vannote et al. 1980, Maranger et al. 2005, Battin et al. 2008). Much of the terrestrially-derived OM delivered to aquatic systems is thought to be relatively well-processed and refractory by the time it reaches the aquatic system, yet it constitutes a substantial resource subsidy fueling riverine bacterial communities (Maranger et al. 2005, Battin et al. 2008). Indeed, most large riverine systems are net-heterotrophic, meaning more OM is processed than is produced locally (Maranger et al. 2005, Battin et al. 2008). Bacteria use OM from both allochthonous and autochthounous sources for two primary functions: respiration and production (del Giorgio and Cole 1998). Bacterial respiration (BR) is the use of C for cellular maintenance and metabolism, while bacterial production (BP) is the use of C in cell growth and division (del Giorgio and Cole 1998). The ratio of BP to the total C processed is the bacterial growth efficiency (BGE = BP/[BP+BR]; del Giorgio and Cole 1998, Maranger et al. 2005). Many aquatic systems exhibit positive relationships between BP-BR and BP-BGE, and BP is typically the more dynamic variable in these systems (del Giorgio and Cole 1998, Maranger et al. 2005). Within system rates of riverine BR have been found to be spatially stable and are generally greater than rates of BP (Maranger et al. 2005).

Although the relationship between OM and bacterial metabolic function has been explored (Maranger et al. 2005, del Giorgio et al. 2006), the relationship

between nutrient dynamics and bacterial metabolic function in riverine systems remains largely unstudied (Ochs et al. 2010, Williams et al. 2012). Bacterial production rates have been linked to the quantity and quality of dissolved organic carbon (DOC) in both lake and stream ecosystems (Bergström and Jansson 2000, Ochs et al. 2010, Roiha et al. 2012) as well as to nutrient concentrations in riverine systems (Ochs et al. 2010). However, the relationship between BR and nutrients is rarely addressed (Preen and Kirchman 2004, Vidal et al. 2011). Bacterial respiration appears to be co-limited by C and P in oligotrophic lake systems (Vidal et al. 2011). Finally, BGE has been shown to be related to the C:N ratio of organic matter as well as N and P concentrations (del Giorgio and Cole 1998).

Because of small size, limited variation in morphology, and lateral gene flow mechanisms, the biodiversity of bacterial communities has been difficult to evaluate (Green and Bohannan 2006, Dinsdale et al. 2008). Recent developments in the areas of bioinformatics have greatly improved our ability to examine microbial communities and the environmental factors that drive patterns of in the abundance and distribution of bacterial groups within microbial communities (Kirchman et al. 2005, Xu 2006). It is thought that within the large divisions of bacteria there are some broad patterns of biogeography (Glöckner et al. 1999, Kirchman et al. 2005). For example, α-proteobacteria have been found to be more abundant in ocean and estuarine systems, while β-proteobacteria are thought to be more abundant in pelagic freshwater systems (Kirchman et al. 2005, Garneau et al. 2006). At smaller regional- and ecosystem scales, linking patterns in community composition to physicochemical parameters has proven more difficult. Gao et al. (2005) found that β- and γ-proteobacteria were correlated with DOC, NO_3^-, and OM in riverine sediments, whereas Barlett and Leff (2010) found very weak response of these groups to nutrients in wetland mesocosms. Additionally, Rubin and Leff (2007) found a positive correlation between β- and γ-proteobacteria and NO_3^-, yet a strongly

negative correlation with DOC in riverine biofilms. In pelagic communities, most studies have addressed longitudinal changes in bacterial community composition as one travels downstream along the mainstem of a river, and few have investigated the patterns in multiple catchments (Kirchman et al. 2005, Winter et al. 2007). A better understanding of the linkage between bacterial community composition and nutrient conditions would help elucidate the forces that structure bacterial communities.

The linkage between bacterial community composition and bacterial metabolic function (e.g., BP, BR, BGE) is tenuous (Findlay 2010). There is some evidence that bacterial community diversity is related to bacterial metabolic function, but the relationship is weak (Langenheder et al. 2005, Lindström et al. 2010). Additionally, many of the diversity assessment methods do not directly identify taxonomic composition (Findlay 2010). Few studies have addressed the relationship major groups of bacteria have with bacterial metabolic function, especially in riverine systems (Kirchman et al. 2004, Kirchman et al. 2005). In the Hudson River, BP was correlated with the proportional abundance of α-proteobacteria (Kirchman et al. 2004), whereas in the Delaware Estuary, BP was correlated with the proportional abundance of β-proteobacteria and high G+C gram-positive bacteria (Kirchman et al. 2005). Given the broad diversity of members in the bacterial divisions, a better understanding is needed about the interactions between environmental conditions, bacterial function, and the patterns of community composition.

In the study presented here, we assessed the relationship between measures of bacterial metabolic function and environmental conditions in order to better understand the relationship between physicochemical conditions and microbial nutrient processing in riverine ecosystems. To do this, we assessed bacterial metabolic function, pelagic bacterial community structure, and physicochemical

conditions in the Brazos River (TX) watershed. We determined if there were landscape-level patterns in bacterial metabolic function and community composition, and assessed the degree to which they were related to physicochemical conditions. Further, we determined if patterns in bacterial community composition could be used to better understand the patterns of bacterial metabolic function. Our overall goal was to determine if the biogeographical patterns of bacterial function and community composition provide evidence as to the nutrient dynamics influencing bacterial communities on a landscape scale. Previous work has identified substantial environmental and nutrient gradients across the Brazos watershed (Becker et al. *in revision*), and we hypothesized that there would be patterns of bacterial function related to the delivery of nutrients and organic matter along these gradients. We additionally hypothesized that there would be shifts in bacterial community composition; driven at least in part by physicochemical conditions, and that these would transfer into patterns of bacterial metabolic function.

Methods

Study Area

The Brazos River spans a distance of 2060 river km from its source near the Texas – New Mexico border to the Gulf of Mexico, and is the 11th longest river in the United States. The watershed is ~116,000 km^2, and spans eight distinct ecoregions (Griffith et al. 2004, Zeug and Winemiller 2008, Vogl and Lopes 2009). Our study area consisted of the lower ~⅓ of the watershed, covering an area of ~41,000 km^2 (Fig. 3.1). Previous work on the Brazos River watershed has identified substantial gradients in environmental conditions in the watershed that are the main driver of nutrient and water quality conditions (Vogl and Lopes 2009, Becker et al. *in revision*). The main-stem portion of the river in the study area is

free of impoundments, however the river upstream and the major tributary sub-watersheds in the study area are regulated by dams (Zeug and Winemiller 2008). Land use across the entire lower Brazos watershed is predominantly agriculture and grazing (Zeug and Winemiller 2008, Becker et al. *in revision*); however, the individual sub-watersheds have distinct patterns of land use and environmental gradients (Becker et al. *in revision*). For this study, we sampled 16 sites across the lower Brazos watershed, primarily along the major tributaries, including the Lampasas, Little, and Navasota Rivers, and Yegua Creek (Fig. 3.1). Additionally, we sampled four sites along the mainstem of the Brazos River. Detailed site location information is presented in Becker et al. (*in revision*).

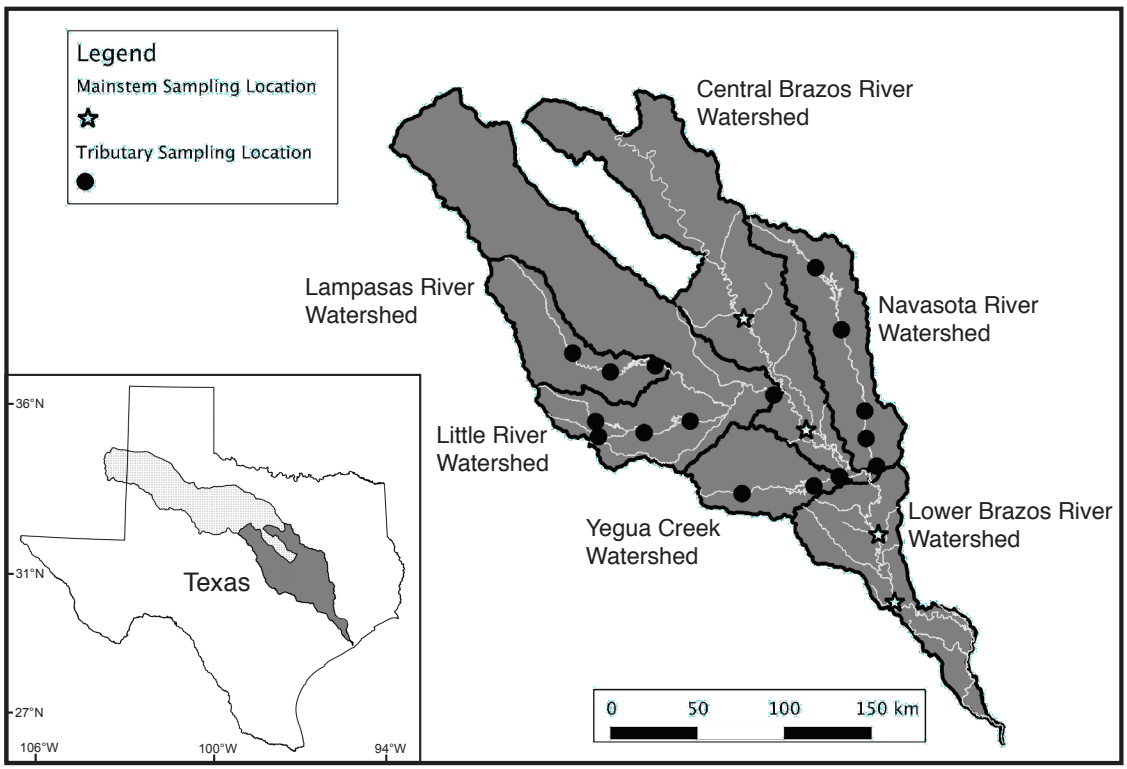

Fig. 3.1. Stream sampling locations and study catchments in the Brazos River watershed in Texas. Inset shows the entire Brazos River watershed. Light stippling indicates the upper Brazos watershed; grey area indicates focus of the present study.

Stream Sampling and Laboratory Analyses

Nutrient and bacterial metabolic function samples were collected from all sites for three field seasons of 2008-2009. Spring sampling occurred March – May 2008, summer sampling occurred June – August 2008, and winter sampling occurred November 2008 – January 2009. Detailed descriptions of sampling and processing procedures for the nutrient data is in Becker et al. (*in revision*). Briefly, water for nutrient analyses was collected as surface grab samples in acid-washed 2-L brown Nalgene™ bottles. Bottles were rinsed 3x with site water prior to sample collection. Bottles were placed in coolers on ice until processed in the lab within 24 – 48 h of collection. In the lab, samples were immediately analyzed or divided into subsamples and preserved for future analysis. Water analyses included in this study were soluble reactive phosphorous (SRP), particulate phosphorous (PP), dissolved nitrate (NO_3^-), dissolved ammonium (NH_4^+), particulate N (PN), dissolved organic carbon (DOC), particulate carbon (PC), non-volatile suspended solids (NVSS), chlorophyll *a* (chl *a*) dissolved oxygen (DO), temperature (temp), and pH. Additionally, to assess the potential for nutrient limitation, we calculated the seston molar C:N, C:P, and N:P ratios, as well as molar DOC:DIN (DIN = NO_3^- + NH_4^+), DOC:SRP and DIN:SRP. For data analysis, the two duplicate samples for each analyte from each site were averaged. A reported value of half the detection limit was used when values were below detection. These parameters were chosen for inclusion in this analysis (1) in order to reduce multicollinearity between predictors, (2) because they had a wide range of response to physiographic and land-use parameters (Becker et al. *in revision*), and (3) because they represent the major groupings of nutrient conditions in the Brazos River watershed (Becker et al. *in revision*).

Whole community bacterial production (BP) was measured using the microcentrifuge ^{3}H-leucine method on six 1.5 ml aliquots of whole (unfiltered) water from each site (Smith and Azam 1992, Caston et al. 2009). The same brand and type

of microcentrifuge tube was used throughout the study (Pace et al. 2004). All tubes received ^{3}H-leucine, while two tubes received 50% cold trichloroacetic acid (TCA) at the beginning of incubation, and the rest received 50% cold TCA after 45-60 min of incubation in the dark at *in situ* river temperatures. ^{3}H activity was measured on a Beckman LS 60001C scintillation counter and bacterial production was expressed as μg C L^{-1} h^{-1}.

Whole community bacterial respiration (BR) was estimated through the use of biological oxygen demand (BOD) incubations (Roland et al. 1999, Kritzberg et al. 2005)} on both whole-water (unfiltered) and water filtered through ashed Pall A/E glass fiber filters (1μm nominal pore size, thus <1μmBR). Whole-water BR estimates include both free-floating and suspended particulate-attached bacteria, while <1μmBR is an estimate of the putatively non-attached or free-floating bacteria. To estimate BR, we conducted relatively short-term (48-72 h, depending on water temperature, with winter, low-temperature samples being incubated for longer) incubations in 60-mL Whatman BOD bottles with glass stoppers. Six replicate bottles each were filled with whole water or filtered water. Initial DO concentrations were measured in duplicate bottles, leaving 4 bottles to incubate. Dissolved oxygen concentrations were measured using a modified spectrophotometric Winkler method (Roland et al. 1999). The remaining replicate bottles were incubated in the dark at *in situ* river temperature. After incubation, bottles were removed and DO was determined. Oxygen consumption (μg O_2 L^{-1} h^{-1}) was calculated as the difference between initial and final DO. Oxygen consumption values were converted to C respired (μg C L^{-1} h^{-1}) based upon a respiratory quotient of 1 (del Giorgio et al. 2006). We acknowledge that whole water respiration may be an overestimate of BR, however it represents a maximum rate and there are methodological issues with the separation of BR from total planktonic respiration (Vidal et al. 2011) and the use of filtered water to determine BR is known to result in substantial underestimates

(del Giorgio et al. 2006). Although filtration has been shown to remove free-floating bacteria, other authors have estimated that less than 40% (and likely 10 – 20%) of the bacterial community is particle attached (del Giorgio and Pace 2008). This appears to be an underestimate for the Brazos River, as the reduction by approximately 65% in BR of filtered water suggests that much of the active bacterial community in the Brazos River is attached to particles. Measurements of whole-water BR were coupled with BP estimates in order to estimate bacterial growth efficiency (BGE). Bacterial growth efficiency was calculated as BGE = BP / (BP+BR). Additionally, as a measure of the bacterial community's ability to utilize organic carbon (i.e., organic C use efficiency), we calculated BP:DOC (μg-BP mg-DOC^{-1} hr^{-1}; Bergström and Jansson 2000, Lindström et al. 2010).

Bacterial community composition was assessed at all 20 sites during the summer sampling period. Bacterial community composition was numerically quantified with fluorescent *in situ* hybridization (FISH; Rabus et al. 1996, Zarda et al. 1997). Samples were collected in duplicate sterilized 50 mL centrifuge tubes, filled with site water and stored on ice until return to the lab (within 48 hrs). At the lab, tubes were centrifuged at 3000 x g for 1hr at 4°C, and supernatant was removed. The remaining pellet was fixed with 1.5 mL of 4% paraformaldehyde/ phosphate buffered saline (PBS) fixative for 12-24 hrs. Afterwards, the pellet was re-suspended, transferred to autoclaved 2mL centrifuge tubes, washed twice with PBS, and stored in a 1:1 mixture of PBS and ethanol at -20°C (Zarda et al. 1997). For enumeration, all microbial cells were stained with the DNA intercalating dye 4',6-diamidino-2-phenylindole (DAPI), while Cy3-labeled probes were used to enumerate the bacterial divisions (see Table 3.1 for probe specifics, hybridization conditions and references). Groups counted included domains Bacteria, Archaea, α-proteobacteria, β-proteobacteria, γ-proteobacteria, δ-proteobacteria, *Cytophaga-Flavobacter*-like bacteria of the CFB phylum, *Actinobacteria* sub-branch of the Gram-

Table 3.1. In-situ hybridization probes and conditions used to examine the abundance of the major prokaryotic groups in the Brazos River watershed. All counts were co-stained with DAPI. δ-Proteobacteria were costained with both probe SRB385 and SRB-Db.

Phylogenetic Group	Probe	Probe sequence (5'-3')	Position	Formamide %	Reference
Bacteria	EUB338	GCTGCCTCCCGTAGGAGT	16S rRNA, 338-355	30	Amann et al. (1995)
Archaea	ARCH915	GTGCTCCCCCGCCAATTCCT	16S rRNA, 915-934	20	Amann et al. (1995)
α-Proteobacteria	ALF1b	CGTTCGYTCTGAGCCAG	16S rRNA, 19-35	10	Amann et al. (1995)
β-Proteobacteria	BET42a	GCCTTCCCACTTCGTTT	23S rRNA, 1027-1043	30	Amann et al. (1995)
γ-Proteobacteria	GAM42a	GCCTTCCCACATCGTTT	23S rRNA, 1027-1043	30	Amann et al. (1995)
δ-Proteobacteria	SRB385 & SRB-Db	CGGCGTTGCTGCGTCAGG CGGCGTCGCTGCGTCAGG	16S rRNA, 385-402	20	Amann et al. (1992) Rabus et al. (1996)
Cytophaga-Flavobacterium	CF319a	TGGTCCGTGTCTCAGTAC	16S rRNA, 319-336	35	Amann et al. (1995)
Actinobacteria	HGC69a	TATAGTTACCACCGCCGT	23S rRNA, 1901-1918	20	Amann et al. (1995)
Bacillus	LGCa,b	YSGAAGATTCCCTACTGC	16S rRNA, 354-371	20	Meier et al. (1999)

positive bacteria with high DNA G+C content (GPB-HGC) phylum, and the *Bacillus* sub-branch of the Gram-positive bacteria with low DNA G+C content (GPB-LGC) phylum of bacteria (Amann et al. 1992, Amann et al. 1995, Rabus et al. 1996, Meier et al. 1999).

For slide application, the sample was sonicated for 5 seconds and a subsample was sequentially dispersed in 0.1% sodium pyrophosphate to a 1-25% sample concentration, so that there were approximately 100 DAPI-stained cells per microscope field (0.01 mm^2). Of this diluted sample, 10 µl was dispersed into the well of a gelatin coated 8-well slide and dried at 35°C. To improve cell permeability samples were treated with 10 µl 1% lysozyme for 30 min at room temperature (Zarda et al. 1997). To hybridize the probes, 9 µl of hybridization buffer, 1 µl of the probe or probe mix (50 ng), and 1 µl of DAPI solution (200 ng) was applied to each sample, and incubated at 42°C for 3 h in a humid chamber. For counting, slides were mounted with Citifluor™ AF1 solution (Citifluor Ltd.) and examined with a Nikon Eclipse 80i microscope (Nikon Instruments), fitted for epifluorescence microscopy with a mercury lamp (Nikon; X-Cite™ 120) and two filter cubes, UV-2E/C (Nikon; EX340-380, DM400, BA4435-485, for DAPI detection) and Cy3 HYQ (Nikon; EX535/50, DM565, BA610/75, for Cy3 detection). For each site, 20 fields covering 0.01 mm^2 were haphazardly selected from each slide well hybridized with each probe, and cell counts were converted to the average cells per ml in the original

sample. For analysis, each replicate was counted and the counts from each were averaged.

Data Analysis

We initially assessed the relationships between the various measures of bacterial metabolism (BP, BR, <1μmBR, BGE, BP:DOC) through the use of ordinary least square regression on $\log_{10}(x)$ or $\log_{10}(x + 1)$ transformed data, except BGE, which was logit transformed to best meet the assumptions of normality (Warton and Hui 2011). To assess if there were broad spatial and temporal patterns in bacterial metabolism across the Brazos drainage we used mixed-effect repeated measures analysis of variance (rm-ANOVA), with watershed as a fixed effect, and the seasonal samples as a random effect, nested within the watersheds. In order to assess the relationship between variation in physicochemical conditions and bacterial metabolic function we used redundancy analysis (RDA), a constrained ordination extension of principal component analysis (PCA) that allows for the selection of predictor and response datasets (Legendre and Legendre 2012). This analysis allowed us to assess the relationships between multiple predictors and response variables, in what is an extended multivariate regression framework (Legendre and Legendre 2012). Bacterial function data (BP, BR, <1μmBR, BP:DOC) were $\log_{10}$ transformed (Legendre and Legendre 2012), while BGE data was logit transformed to best meet the assumption of normality in response data (Warton and Hui 2011). For RDA, both predictors and response variables were *z*-score transformed and significance of the ordination was assessed through permutation tests (n = 1000; Borcard et al. 2011, Legendre and Legendre 2012). For the RDA models, we present the first two axes corrected by the R^2_{adj}, a more conservative measure of explanatory power than the commonly reported "proportion of inertia explained" (Peres-Neto et al. 2006, Borcard et al. 2011).

In order to assess the potential relationships between bacterial community abundance (as detected by the DAPI and EUB probes) and bacterial function we used ordinary least square regression on $\log_{10}$ transformed data (except BGE, which was not transformed as it was normally distributed). To assess the relationship between bacterial community composition and the various measures of bacterial metabolic function (BP, BR, etc) we used a backwards model selection procedure based on the minimum Akaike's information criterion corrected for small sample size (AICc; Burnham and Anderson 2004). Bacterial production was the only variable that had a significant relationship to total bacterial abundance and the relationship was unimodal (see Results below), thus, for this analysis we converted the bacterial community composition into proportional abundances of each bacterial group out of the total bacteria abundance determined with the EUB probe. Archea were excluded from this analysis because they rarely made up > 1% of the total bacterial abundance (as determined by DAPI counts). Bacterial community composition was used as the predictor dataset and data were not transformed because most data were normally distributed. In this analysis, we again used the bacterial metabolic function data from the summer sampling season. The same transformations were applied as in the community composition-metabolic function analysis, except that BGE was not transformed as it was normally distributed for the summer sampling (Legendre and Legendre 2012). Because the gradient length (i.e., the difference between the high and low proportional abundances) was < 3 times the standard deviation, we were justified in using RDA (Ramette 2007).

Finally, in order to assess the relationship between environmental conditions and bacterial community composition we used a combination of hierarchical clustering and linear discriminant analysis (LDA; Borcard et al. 2011, Legendre and Legendre 2012). In this context, hierarchical clustering identified groups, or "clusters", of sites that were more similar in their bacterial community composition

to sites within a group than they were to sites in other groups, thus minimizing the sum of squares differences within the groups (Ramette 2007, Legendre and Legendre 2012). Hierarchical clustering using Ward's minimum variance was performed on $\log_{10}$-transformed abundance data for the bacterial groups (Borcard et al. 2011); abundance data was transformed to reduce the influence of several sites with extremely high bacterial counts. Data were not further transformed because they were of the same scale and there was no issue with double-zeros in the species matrix (Borcard et al. 2011, Legendre and Legendre 2012). To determine if nutrient conditions could be correlated with the identified bacterial clusters, we used LDA to test if physicochemical conditions were associated with the same bacterial groupings (Ramette 2007, Legendre and Legendre 2012). Nutrient data was *z*-score transformed prior to analysis so that the magnitude of all variables was consistent (Ramette 2007). To determine the LDA model we used a backwards selection procedure where nutrients were sequentially removed from the analysis until all remaining predictors were significant ($\alpha = 0.05$; Legendre and Legendre 2012). To assess how well the LDA fit the bacterial community composition derived groups from the hierarchical clustering analysis, a jackknife, leave-one-out cross-validation procedure was used to test for misclassifications (Borcard et al. 2011). Although the analysis of the clustering supported 4 groups of sites, 7 sites were misclassified (35%), thus we opted to run the analysis with 3 clusters, which only misclassified 2 sites in the jackknife cross-validation (Borcard et al. 2011). To assess the associations between the $\log_{10}$ transformed abundance of each group of bacteria and the nutrient predictor variables identified in the LDA, we performed RDA on the *z*-score standardized and transformed nutrient and bacterial abundance datasets (Legendre and Legendre 2012). Significance of the RDA was assessed by permutation tests (Legendre and Legendre 2012). In the present study, RDA was performed using the 'vegan' package in R (Oksanen et al. 2012, R Core Team

2013). Hierarchical clustering was performed using R (R Core Team 2013), and the LDA was performed in the 'MASS' package in R (Venables and Ripley 2002). All univariate statistics and the model selection procedure for the LDA were performed using JMP 10.0 (SAS, Inc., Cary, North Carolina) platform.

Results

Relationships Between Bacterial Metabolism and Nutrients

Across all the sites and seasons, the range of BP in the Brazos River watershed was 0.07 – 6.07 μg C L^{-1} h^{-1} (1.70 ± 1.28 μg C L^{-1} h^{-1}, mean ± s.d.). The range of BR was 0 – 30.4 μg C L^{-1} h^{-1} (8.68 ± 7.28 μg C L^{-1} h^{-1}). The range of <1μmBR was 0 – 11.6 μg C L^{-1} h^{-1} (2.70 ± 3.15 μg C L^{-1} h^{-1}). The range of BGE was 0.005 – 0.87 (0.23 ± 0.16). The range of organic C use efficiency (BP:DOC) was 0.01 – 6.61 (0.69 ± 1.17 μg-BP mg-DOC^{-1} h^{-1}). Organic C use efficiency was highly skewed, primarily by multiple sites with below detection level DOC during the spring sampling. There was a positive relationship between BP and BR (R^2 = 0.25, p < 0.001), as well as a weak relationship with <1μmBR (R^2 = 0.09, p < 0.020), and there was a negative relationship for both BR and <1μmBR with BGE (R^2 = 0.50, p < 0.001 and R^2 = 0.09, p < 0.017, respectively). However, BP and BGE were unrelated (p = 0.142). Both measures of bacterial respiration (BR and <1μmBR) were significantly correlated (R^2 = 0.31, p < 0.001) and on average <1μmBR was 35% of BR, although the variability was proportionally higher (coefficient of variation [CV] = 117% for <1μmBR v. 84% for BR). For comparison, the relationship between BP and <1μmBR was significant but weak (R^2 = 0.09, p = 0.02). There were no consistent watershed effects for any measures of bacterial metabolic function (all p > 0.167) and season explained a substantial proportion of the variation in the data, ranging between 24.2% (for BP) and 43.8% (for BR).

The RDA to assess the relationship between bacterial metabolic function and

nutrient conditions was significant (R^2_{adj} = 0.40, p < 0.001; Fig. 3.2), and the first two RDA axes accounted for 34% of the variation in bacterial metabolic function data. The first RDA axis (RDA1) explained 20% of the variation in the data and largely represented a gradient of particulate nutrients and temperature (with negative loadings) to dissolved fraction nutrients and DO (with positive loadings) across the sampling sites in the drainage. The second RDA axis explained 14% of the variation in the data and represented a gradient of sites elevated DIN:SRP and seston C:P (with negative loadings) to elevated DOC, NH_4^+, SRP and DOC:DIN (with positive loadings). Bacterial production, BR, and <1µmBR were positively correlated with elevated levels of particulate nutrients, as well as an elevated sestonic C:N

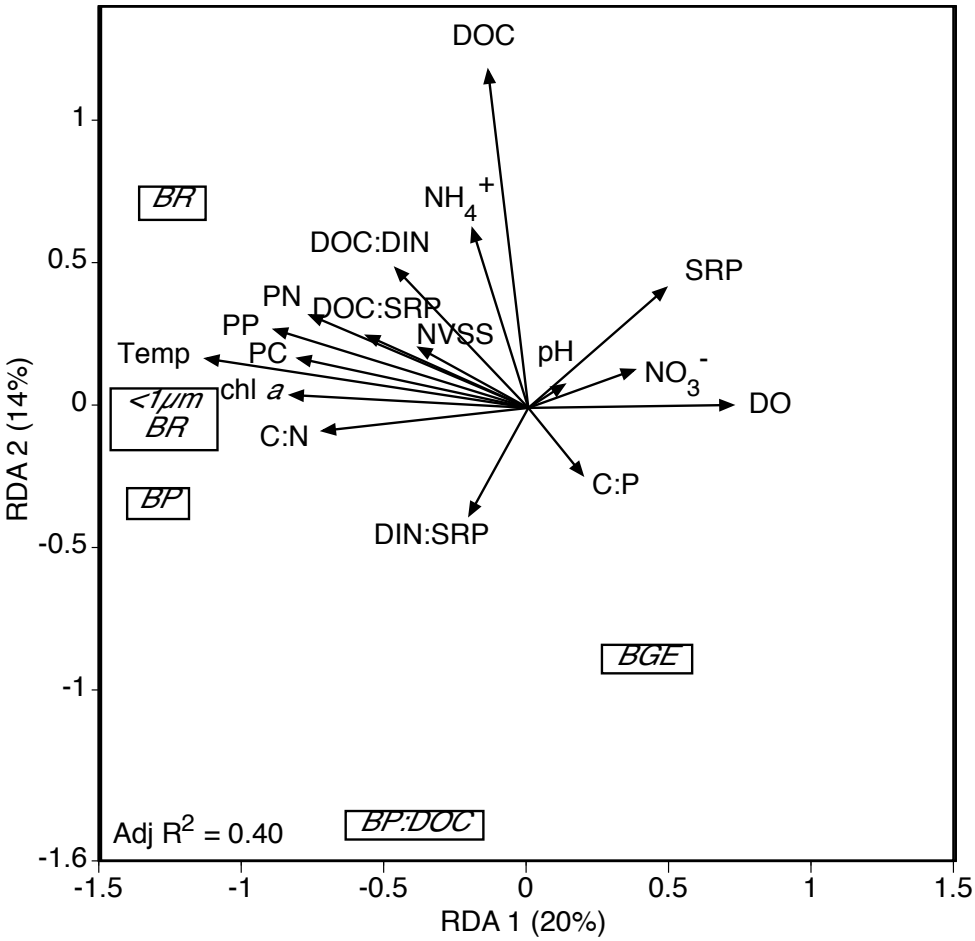

Fig. 3.2. Redundancy analysis plot of the relationships between nutrient conditions and bacterial environmental function. Functional response variables are boxed and italicized. Abbreviations are consistent with the text.

and negatively correlated with DO, SRP, and NO_3^- concentrations. Bacterial growth efficiency and the organic C use efficiency were positively correlated with the DIN:SRP and seston C:P, and negatively correlated with DOC, NH_4^+, and SRP.

Relationships of Bacterial Abundance and Community Composition with Metabolic Function

Total microbial abundance estimated by DAPI counts ranged from 1.2 x 10^6 – 2.6 x 10^7, while total bacterial abundance estimated by EUB probe counts ranged from 6.8 x 10^5 – 1.2 x10^7. Bacterial abundance estimates by DAPI and EUB counts were highly correlated (R^2 = 0.99, p < 0.001) with EUB counts averaging approximately 47.5 ± 1% of the DAPI estimates. Given the high correlation between the community abundance estimates, we used the estimated abundance from the EUB probe in any subsequent analyses. The only measure of bacterial metabolic function that was significantly correlated with bacterial abundance was BP, which had a marginally significant second-degree polynomial (unimodal) fit (R^2 = 0.30, p < 0.049; Fig. 3.3). This indicates that BP did not scale linearly with bacterial

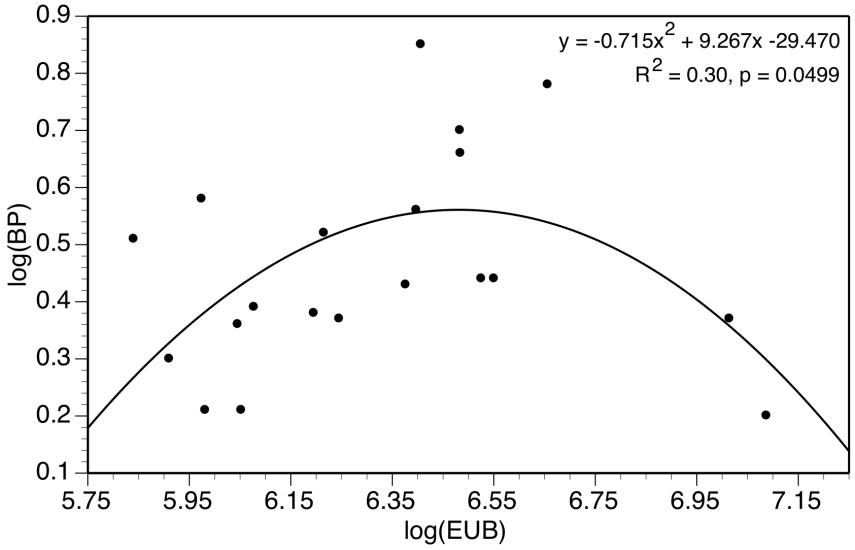

Fig. 3.3. Univariate relationship between bacterial population estimates and bacterial production. Both variables have been log10x transformed. EUB = bacterial population estimate using the EUB338 probe (Table 3.1). BP = bacterial production.

abundance, and that at very high abundances BP rates were restricted.

Model selection using minimum AICc criteria on bacterial proportional abundance indicated that bacterial metabolic function only responded weakly to changes in bacterial community composition (Table 3.2). Bacterial respiration was negatively correlated with the proportion of *Actinobacteria* (R^2 = 0.23, p = 0.0303). Putatively free-floating bacterial respiration (<1μmBR) had a more complex relationship with community composition (R^2 = 0.67, p < 0.001), having a positive relationship with the proportion of α-proteobacteria and negative relationships with γ-proteobacteria and *Actinobacteria*. The other measures of bacterial metabolic function had non-significant relationships with the proportional bacterial community composition.

Landscape Patterns of Bacterial Community Composition

Based upon the hierarchical clustering, sites partitioned into a group of sites located in the upstream/northwestern portion of the drainage, and a largely downstream/southeastern grouping of sites; with the two most downstream mainstem Brazos River sites separated out into their own group, largely due to bacterial abundances that were an order of magnitude greater than at any of the other sites. The stepwise variable selection process in the subsequent LDA indicated that 9 nutrient variables were significant in explaining the differences between the three groups of sites (all p < 0.05, Table 3.3). The first LDA axis (LDA1) separated

Table 3.2. Results of multiple regression analyses testing the ability of the proportional abundances in the bacterial community compostions to predict measures of bacterial ecosystem function. The models with the lowest AICc score are listed. BMF = bacterial metabolic function. CR = coefficient of regression for the selected predictors. BP = bacterial production. BR = Bacterial respiration. <1μmBR = bacterial respriration in filtered water. BGE = bacterial growth efficiency. Bold indicates p <0.05. na = no model was significant.

BMF Response	Best Model	AICc	R^2_{adj}	*CR*	*p*
logBP	na	na	na	na	na
logBR	*Actinobacteria* (-)	5.8	0.24	-3.53	**0.0303**
log<1μmBR	x-proteobacteria (+), γ-proteobacteria (-), *Actinobacteria* (	4.3	0.25	3.32, -6.83, -4.2	**0.0004**
BGE	*Actinobacteria* (+)	-36.3	0.14	0.89	0.1086
logBP:DOC	β-proteobacteria (-), *Cytophaga-Flavobacterium* (-)	-32.2	0.24	-0.28, -0.79	0.0931

Table 3.3. Results of the stepwise variable selection process for inclusion in the LDA to relate differences in the bacterial commuity to environmenal conditions. Values in bold are significant at $p < 0.05$ and were included in the LDA model. Abbrevaitions are consistent with the text. DIN:SRP was collinear with DOC:DIN and was excluded from the analysis.

Nutrient Predictor	*F*-ratio	*p*
SRP	28.79	**<0.001**
PP	49.33	**<0.001**
NO_3^-	25.73	**<0.001**
NH_4^+	0.67	0.538
PN	75.6	**<0.001**
DOC	0.06	0.942
NVSS	24.76	**<0.001**
PC	167.95	**<0.001**
C:N	0.5	0.624
C:P	67.18	**<0.001**
N:P	0.391	0.689
Temp	2.821	0.118
DO	5.083	**0.033**
Chl a	0.806	0.48
DOC:DIN	0.264	0.774
DOC:SRP	24.92	**<0.001**
DIN:SRP	--	--

the site groupings on a gradient of sites with elevated PP, PN, seston C:P and NO_3^- (with negative loadings) to sites with elevated PC, SRP, DOC:SRP, and NVSS (with positive loadings; Fig. 3.4). Spatially within the Brazos basin, the sites representing the downstream tributaries had the most negative loadings, the upstream and headwaters sites had loadings near the origin, and the downstream mainstem sites extremely high positive loadings. Along the second LDA axis (LDA2) site groupings separated along a gradient of elevated PN, PP, seston C:P, and NVSS (with negative loadings) to elevated PC, SRP, and DOC:SRP. This axis separated the sites in the upper drainage from sites in the lower drainage.

The abundances of individual groups of bacteria were largely associated with increases in particulate matter along RDA1 (Fig. 3.5). However, some groups exhibited some separation along RDA2. *Actinobacteria* were associated with increased SRP, and decreased DOC:SRP. Conversely, β-proteobacteria cells were more associated with increased NO_3^- concentration and weakly correlated with

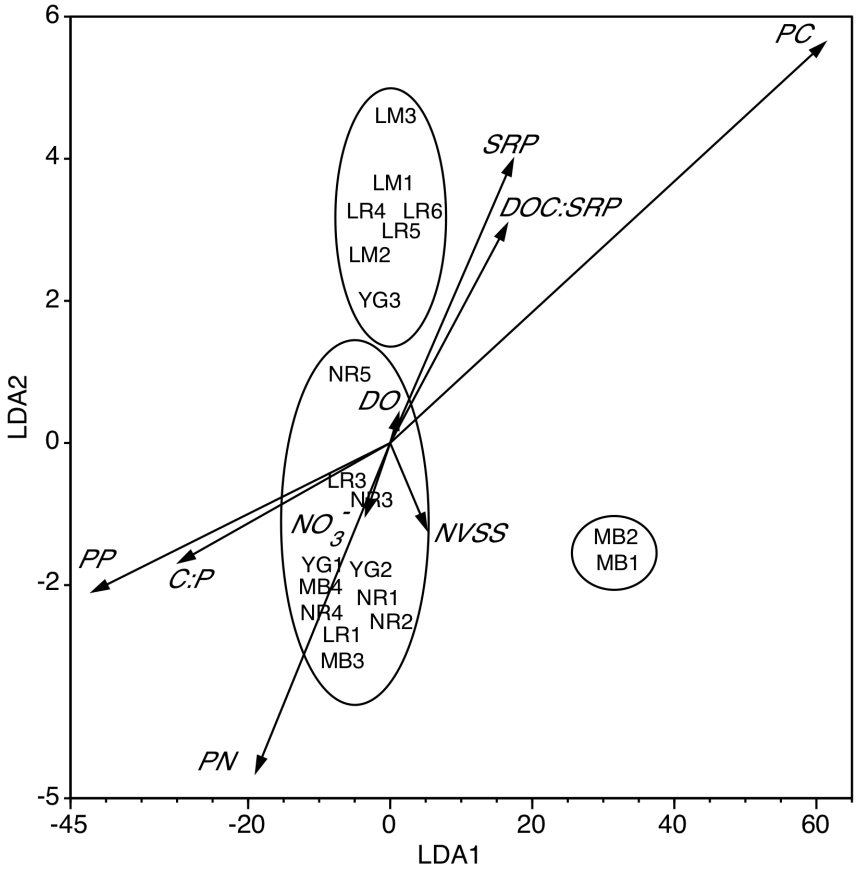

Fig. 3.4. Results of the linear discriminant analysis. Nutrient variables are italicized. The three groupings determined by hierarchical clustering of the bacterial community are circled. Some sites have been moved slightly to improve readability of the figure, however the interpretation does not change.

decreased sestonic C:P. Proportionally, β-proteobacteria and *Actinobacteria* were the most common divisions of bacteria; together making up approximately 24% of the domain Bacteria-identified cells.

Discussion

Relationships Between Measures of Bacterial Metabolic Function

In the study presented here, we found that there was a significant correlation between BP and BR. In the Brazos River, BR rates were larger (on average ~5×) and more dynamic than BP. This is in contrast to many other aquatic systems, where BP has been the more dynamic variable (del Giorgio and Cole 1998, Maranger et al. 2005). It is thought that rates of BP are correlated with more labile forms of

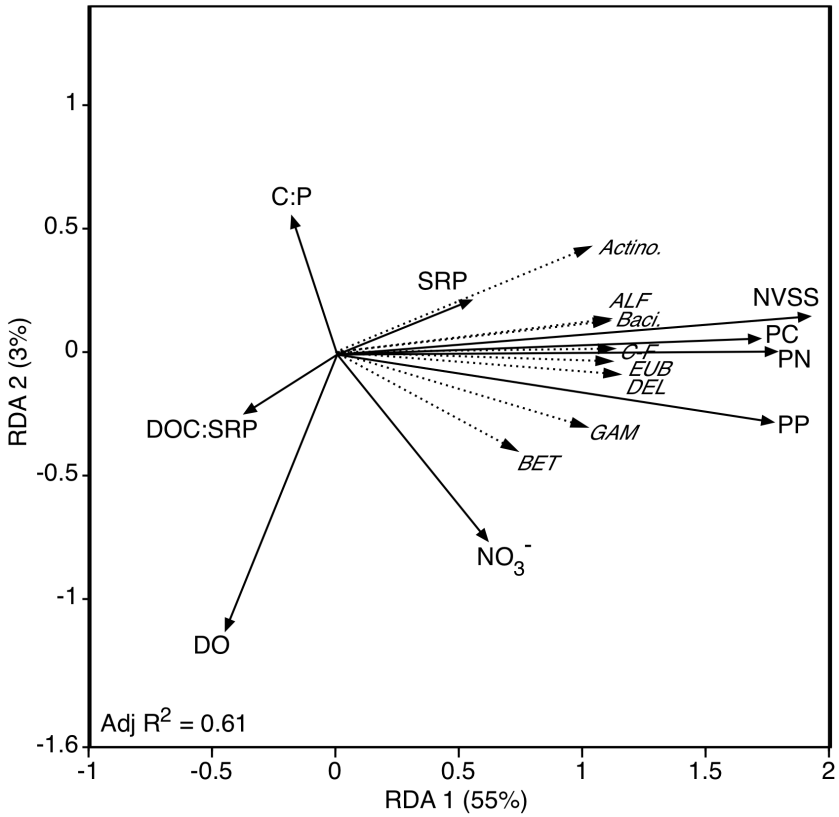

Fig. 3.5. Redundancy analysis plot of the relationships between nutrients and the bacterial community composition. Nutrient abbreviations are consistent with the text. Bacterial groups are italicized and represented by dashed vectors. EUB = Domain bacteria; Actino. = Actinobacteria; ALF = α-proteobacteria; BET = β-proteobacteria; GAM = γ-proteobacteria; DEL = δ-proteobacteria; C-F = Cytophaga-Flavobacter; Baci. = Bacillus.

organic C, often from primary producer exudates (Kritzberg et al. 2005, Maranger et al. 2005), and this pattern was also observed across the Brazos River watershed as indicated by the positive relationship between BP and chl *a* (Fig. 3.2). However, both measures of BR were also associated with elevated chl *a*, and neither BP nor BR was correlated with DOC, further suggesting the relatively greater importance of autochthonous C in overall bacterial community metabolism (Vidal et al. 2011). The pattern of primary production supporting bacterial C metabolism has been suggested for BP (Kritzberg et al. 2005). However, this has not been thoroughly tested for BR, but has been seen in some estuarine systems (Preen and Kirchman 2004, del Giorgio et al. 2006). If autochthonous production were the main C source supporting both BP and BR, it could explain this pattern, although one would expect

to observe lower BGE under these conditions (Vidal et al. 2011). Additionally, DOC levels are typically in the range thought to indicate net-heterotrophy (>5 mg L^{-1}), which would necessitate a reliance on allochthonous C (Westhorpe et al. 2010). It is likely that DOC supports a baseline BP and BR, but that the variability in each is controlled by autochthonous production (Kritzberg et al. 2005).

Bacterial growth efficiency was within the range of values seen in other riverine systems (del Giorgio and Cole 1998, Maranger et al. 2005, del Giorgio et al. 2006), but the relationships between BP, BR, and BGE in the Brazos drainage were different than those previously observed in other aquatic systems (del Giorgio and Cole 1998, Maranger et al. 2005). It is often assumed that there is a positive relationship between BGE and BP, however in the Brazos River, there was no significant relationship between BP and BGE due to the fact that BR accounted for a much larger fraction of C consumption than BP and was relatively more variable than BP. Bacterial growth efficiency in the Brazos drainage was negatively correlated with both DOC and NH_4^+, and it has been suggested that limits on BGE are complex and there can be co-limitation by both C availability and inorganic nutrients (del Giorgio and Cole 1998, Maranger et al. 2005). Experimental work in oligotrophic boreal lakes has shown co-limitation of BGE by labile-C and dissolved-P (Vidal et al. 2011); however co-limitation by C and P seems unlikely in the agriculturally-dominated Brazos River watershed, given the relatively high SRP concentrations across the drainage (198 ± 400 μg L^{-1}) and the somewhat negative relationship between BGE and chl *a* (Fig. 2). A more likely explanation for the patterns we observed in the Brazos River watershed is that when nutrients are non-limiting to BP, BGE has a negative relationship to temperature (Berggren et al. 2010). Under these conditions BR would increase more relative to BP as temperature increased due to increased metabolic maintenance costs, thus reducing BGE (Berggren et al. 2010).

The ratio of BP:DOC in aquatic ecosystems can be thought of in two ways: (1) as a measure of the ability of the bacterial community's ability to utilize the C resources in an ecosystem (Lindström et al. 2010), or (2) as an indication of the bioavailability and lability of DOC in an ecosystem (Bergström and Jansson 2000). In the Brazos River watershed, it is apparent that the more variable component of this metric is the DOC concentration (CV = 76% for BP and 94% for DOC). Additionally, the RDA to assess the influence of physicochemical conditions on bacterial metabolic function indicated a strong negative correlation between DOC and BP:DOC, and an orthogonal (non-significant) relationship between BP and DOC concentration. The lack of a positive relationship between BP and DOC indicates that the DOC pool in the Brazos River watershed is likely to be largely composed of allochthonous and relatively refractory C (Bergström and Jansson 2000, Kritzberg et al. 2005). Additionally, it further confirms the relationship between BP and autochthounous primary production (chl *a*), as BP is apparently not related to the DOC concentration. Likely, BP is responsive to short-term increases in autochthonously-derived and presumably labile DOC from primary producers (Kritzberg et al. 2005).

Bacterial Community Composition and Metabolic Function

Linking patterns of bacterial community structure to patterns of metabolic function has been difficult (Langenheder et al. 2005). Members within the major divisions of bacteria are functionally and metabolically diverse and there is a large amount of functional redundancy in the bacterial community (Langenheder et al. 2005, Lindström et al. 2010). Additionally, bacteria have the ability to persist in resting stages, only coming out of dormancy when environmental conditions are favorable (Jones and Lennon 2010). In the present study, the unimodal relationship between total bacterial abundance and BP was the only clear link between microbial community and metabolic function. The nature of this relationship was influenced

by the two most downstream mainstem river sites that had bacterial counts an order of magnitude higher than any other sites, suggesting that even at relatively high abundances, there are likely physicochemical factors (e.g., nutrients or turbidity) that suppress overall riverine BP rates. When the downstream mainstem sites are excluded from the bacterial density – BP relationship, the density of Domain Bacteria and BP increase linearly ($\log_{10}$-$\log_{10}$ plot; $R^2 = 0.34$, $p = 0.01$). If labile carbon sources are not increasing proportionally with bacterial abundance, the per-cell availability of C may become limiting to BP (Ochs et al. 2010). Particulate material concentrations (presumably more refractory compounds) at these two sites throughout the study was consistently one to two orders of magnitude higher than any other site, while DOC and chl *a* were well-within within the ranges observed of the other sites (Table 3.S1).

In the present study, the abundance of only a few bacterial groups were significantly related to the metabolic function of the bacterial community. Studies explicitly linking bacterial community composition to overall measures of metabolic function are rare in the literature and the results of the few studies that have examined these relationships are equivocal. Kirchman et al. (2005) found that the proportional abundance of β-proteobacteria and *Actinobacteria* were correlated with BP in the Delaware Estuary, and Warkentin et al. (2011) found a positive correlation between β-proteobacteria, γ-proteobacteria, and BP. In contrast, we did not find that the density of any individual group of bacteria was correlated positively or negatively with rates of BP. Studies linking bacterial community respiration rates with community composition are even more rare; we know of only one study addressing the link between community-level BR rates and the bacterial community composition (Warkentin et al. 2011). The authors found a positive correlation between the proportion of γ-proteobacteria bacteria and <1μmBR rates. In contrast, we found a negative correlation with γ-proteobacteria (and *Actinobacteria*) and

<1μmBR in the Brazos River drainage and a positive correlation between <1μmBR and the relative abundance of α-proteobacteria. These results clearly indicate that more study is needed and it is not entirely surprising that the patterns between metabolic function and community composition of broad groupings are equivocal. In addition, the broad functional capacities of different bacterial groups, the nature of the BP – community composition relationship is likely to be dependent on a myriad of factors including the specific bacterial groups present in a given system, the lability of C resources, and the availability of inorganic nutrients (Langenheder et al. 2005, Comte and del Giorgio 2011). Similarly, even bacteria that are not actively growing require carbon for maintenance of cell structures, so the correlation between a specific group of bacteria and community BR rates is likely to be weak, especially within broad groups (del Giorgio et al. 2006, Warkentin et al. 2011).

Responses of Bacterial Community Composition to Environmental Conditions

Unlike the weak relationships between bacterial community composition and community-level measures of bacterial metabolism the present study detected changes in community composition related to spatial variation in physicochemical environmental conditions in the Brazos drainage. Part of the change in bacterial community is related to a general trend of increasing bacterial abundance downstream in the Brazos watershed, opposite of trends seen in other systems (Maranger et al. 2005). Multiple studies have found links between bacterial community composition and changes in nutrient and resource availability (Gao et al. 2005, Comte and del Giorgio 2011) or temperature (Anderson-Glenna et al. 2008). In the Brazos River watershed, the differences in the composition of bacterial community coincided with strong differences in the concentrations of PC and SRP versus PP and sestonic C:P. Differences in physicochemical characteristics across sites are related to large-scale environmental gradients within the Brazos

drainage in which bacterial communities were separated into three distinct groups of similar composition: (1) the upper basin, northwestern group of sites including the Lampasas and upper Little River watersheds, (2) the lower basin, southeastern group of sites including the Navasota, lower Little River, and Yegua Creek watersheds, and (3) the lower mainstem Brazos River sites. The two most downstream mainstem Brazos River sites exhibited greater concentrations of suspended PC and NVSS, supporting the hypothesis that much of the bacteria at these sites, if not attached to suspended particles, were delivered to the river through terrestrial runoff and were potentially more of terrestrial origin, and may not be as metabolically active once moved into an aquatic environment (Ochs et al. 2010). In the Brazos River, PC was highly correlated with SPOM (R^2 = 0.87, p < 0.001). The delivery of terrestrial OM has been show to influence bacterial community composition and metabolic function in lake systems as bacteria appear to use terrestrially-derived OM less efficiently than algal-derived OM (Pérez and Sommaruga 2006). The communities in the upper basin (which also exhibited generally lower bacterial abundances) were associated with elevated levels of SRP and DOC:SRP ratio, while the intermediate abundance populations of the middle drainage were associated with PP, PN and elevated seston C:P. The strong associations with elevated bacterial abundances and particulate nutrients at the two most downstream mainstem sites may be an artifact of high delivery of terrestrial derived cells, especially at high flows (approximately 46 m^3/s at the most downstream site; Crump and Hobbie 2005, Ochs et al. 2010).

In the present study, the two most common groups of bacteria, β-proteobacteria and *Actinobacteria*, exhibited different associations with environmental conditions than the rest of the bacterial community. β-proteobacteria are a common and diverse group, and it is likely that members of this group can survive and remain active across a broad suite of environmental

conditions. However, as a smaller-sized member of the bacteria, β-proteobacteria may be able to better utilize dissolved inorganic nutrients, thereby thriving in a variety of environments (Rubin and Leff 2007). β-proteobacteria were one of the dominant bacterial groups across the Brazos drainage except for the Yegua Creek watershed (where β-proteobacteria made up only 5-9% of the community). The Yegua Creek watershed was characterized by lower NO_3^- and DO, as well as an elevated seston C:P. In contrast, Rubin and Leff (2007) and Gao et al. (2005) both found that β-proteobacteria bacteria numbers were positively correlated with NO_3^- and not associated with SRP. We did not measure salinity, however other authors have associated increases in α-proteobacteria and *Cytophaga-Flavobacterium* group bacteria and decreases in β-proteobacteria and *Actinobacteria* bacteria at sites with elevated salinities (Kirchman et al. 2005, Garneau et al. 2006). We did find a proportional increase in these two groups at the Yegua Creek sites (Table S2), so there may have been a response to salinity or other dissolved solids. Gao et al. (2005) did find a weak correlation between *Actinobacteria* bacteria and chl *a* in stream sediments, however along with β-proteobacteria, *Actinobacteria* are one of two groups that have no univariate correlation with chl *a* in the Brazos River watershed (p = 0.26 and 0.48, respectively). Additionally, there was no clear antagonistic relationship between β-proteobacteria and *Actinobacteria* bacteria, as has been seen in other freshwater systems (Ruiz-Gonzalez et al. 2013). Likely the functional redundancy in the bacterial community made it difficult to assess the relationships between nutrients and community composition at the division level.

Conclusions

In the study presented here, we found that measures of bacterial metabolic function (BP, Br, etc) were correlated, but that the nature of these relationships was not as expected. Most previous studies conducted in other aquatic ecosystems

have observed a positive relationship between BP and BGE (del Giorgio and Cole 1998, Maranger et al. 2005). However, this study and others (Rodibaugh et al., *in prep*) have found that there was no evidence of a relationship between BP and BGE and a negative relationship between BR and BGE. In the present study, BR was the more dynamic variable, and even though BGE was in the normal range for aquatic systems, it was affected by the variability in BR. Bacterial respiration was positively correlated with the biomass of primary producers and temperature, suggesting that in this subtropical riverine system, cell maintenance and growth are highly responsive to the availability of relatively labile C. However, the relationship between community composition of bacteria and their metabolic function was weak. The relative abundances of major groups were not correlated with BP, and only a few groups exhibited a weak relationship with BR. Functional redundancy in the bacterial community as well as the broad nature of the bacterial groups investigated in this study may account for the weak correlations between bacterial community composition and metabolic function (Comte and del Giorgio 2011). We observed changes in bacterial community composition on a landscape scale, although the pattern was in part influenced by bulk changes in bacterial abundance. Although there is mounting evidence in the literature that some groups of bacteria exhibit general patterns of responses to environmental conditions, as a whole, findings are equivocal. In order to improve our ability to understand patterns in community composition in relation to environmental conditions, researchers should use more detailed metagenomic techniques or quantitative probing to investigate within the major divisions of bacteria. Freshwater bacteria are an important component of global C cycle and ultimately, this knowledge will allow us to better understand the unforeseen impacts and long-term implications of human ecosystem modification and climate change.

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Table 3.S1: Physicochemical and bacterial ecosystem function data for the Brazos River Watershed. Summer data was the only data used when assessing bacterial community composition relationships.

		Physicochemical Conditions																	Bacterial Ecosystem Function				
Site Name	Season	SRP	PP	Nitrates	Ammonium	Part N	DOC	NVSS	Part C	C:N	C:P	N:P	Chl a	Temp	DO	DIN:SRP	DOC:DIN	DOC:SRP	BP	BR	<1BR	BGE	BP:DOC
		(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(mg/L)	(mg/L)	(mg/L)	molar	molar	molar	(µg/L)	(°C)	(mg/L)	molar	molar	molar	µg-CL^{-1}h^{-1}	µg-CL^{-1}h^{-1}	µg-CL^{-1}h^{-1}		µg-BP mg-DOC^{-1} h^{-1}
LM1	Spring	7.9	40.9	476.1	33.0	191.2	2.3	13.5	1.8	11.2	112.9	10.3	8.0	20.9	5.9	142.4	5.2	735.7	6.06	4.63	0.00	0.57	2.69
LM2	Spring	35.5	137.2	419.6	52.9	817.8	4.8	103.8	10.3	17.0	193.4	13.2	3.0	21.4	7.0	29.5	11.8	348.9	1.80	8.56	3.18	0.17	0.38
LM3	Spring	60.9	76.2	514.7	41.0	375.1	5.3	39.2	4.1	13.2	137.4	10.9	2.1	22.0	8.3	20.2	11.1	223.2	2.30	9.30	7.29	0.20	0.44
LR1	Spring	134.9	272.0	3398.9	110.6	1248.3	3.4	219.8	16.3	17.6	154.6	10.1	8.5	16.2	8.3	57.5	1.1	64.1	1.28	7.26	1.80	0.15	0.38
LR3	Spring	11.5	90.3	1454.0	45.0	622.7	0.4	35.7	4.2	7.9	120.7	15.2	35.9	18.3	10.6	287.1	0.3	98.3	0.94	2.43	1.08	0.28	2.14
LR4	Spring	64.8	26.3	2725.5	69.0	219.0	0.4	0.1	1.1	6.5	106.9	18.4	3.7	17.9	7.6	95.3	0.2	17.5	1.52	2.98	1.72	0.34	3.45
LR5	Spring	37.5	6.8	166.9	26.6	42.2	0.4	0.1	0.2	7.1	91.4	13.8	0.3	17.4	9.0	11.4	2.7	30.3	0.85	0.27	0.64	0.76	1.93
LR6	Spring	8.3	3.3	242.2	27.1	53.7	0.4	0.1	0.3	6.4	205.0	35.9	0.5	19.6	9.8	71.6	1.9	136.5	0.75	1.44	0.17	0.34	1.70
NR1	Spring	236.5	96.3	1430.7	50.3	259.8	6.9	33.9	1.3	6.4	35.5	6.0	1.9	24.8	5.5	13.8	5.4	74.8	1.09	10.10	0.00	0.10	0.16
NR2	Spring	687.9	116.9	2440.1	59.4	378.3	4.3	38.9	2.0	6.6	44.4	7.2	2.4	24.6	6.2	8.0	2.0	16.1	1.07	14.66	0.73	0.07	0.25
NR3	Spring	31.2	68.8	373.4	39.2	261.6	4.8	29.5	1.3	6.2	48.1	8.4	2.6	24.8	6.9	29.3	13.6	399.1	0.82	4.60	0.00	0.15	0.17
NR4	Spring	22.9	145.3	130.6	43.0	711.2	4.4	149.6	4.8	8.0	85.9	10.8	21.6	20.5	7.8	16.8	29.7	499.5	1.68	1.84	0.00	0.48	0.38
NR5	Spring	64.4	164.2	157.9	98.4	847.2	6.9	79.9	4.8	6.8	75.2	11.4	13.2	23.8	7.0	8.8	31.2	274.7	2.70	11.86	0.00	0.19	0.39
YG1	Spring	191.8	184.4	422.2	65.9	1030.3	13.0	186.1	5.3	6.5	74.1	12.4	2.0	21.3	5.1	5.6	31.0	174.1	1.79	7.45	1.94	0.27	0.14
YG2	Spring	10.1	108.3	109.2	59.6	581.6	5.9	25.4	4.1	4.4	97.5	11.9	17.2	22.1	5.3	36.9	40.7	1505.0	2.61	20.43	4.42	0.11	0.44
YG3	Spring	30.1	111.0	332.4	74.0	854.5	11.4	20.6	3.2	4.4	73.9	17.0	5.6	20.8	7.5	29.9	32.7	977.4	1.38	11.39	7.29	0.11	0.12
MB1	Spring	107.5	916.8	1351.0	30.8	1760.0	5.4	873.5	38.1	25.3	107.1	4.2	8.3	23.5	4.6	28.4	4.6	129.8	1.06	4.35	2.99	0.20	0.20
MB2	Spring	121.1	645.8	1351.0	38.2	2415.8	6.2	579.4	28.8	14.0	115.0	8.3	8.3	24.0	4.3	25.4	5.2	131.9	1.39	5.50	3.83	0.20	0.22
MB3	Spring	23.3	259.9	1147.6	18.0	900.4	0.4	172.7	8.4	11.8	82.9	7.7	22.8	21.0	8.1	110.6	0.4	48.7	2.24	13.04	7.06	0.15	5.09
MB4	Spring	3.9	130.3	672.9	16.7	485.4	0.4	40.4	4.0	9.8	79.2	8.2	10.8	15.8	12.5	390.9	0.7	290.9	2.91	13.04	6.26	0.18	6.61
LM1	Summer	113.1	14.2	449.6	33.3	88.1	2.0	1.0	0.7	9.8	122.5	13.7	0.6	24.2	5.6	9.4	4.8	45.4	0.98	4.08	3.60	0.19	0.49
LM2	Summer	112.6	27.5	15.0	40.5	202.6	2.2	11.3	2.5	16.0	238.3	16.3	2.7	25.3	6.5	1.1	46.0	50.1	2.83	5.23	2.28	0.35	1.29
LM3	Summer	109.9	15.2	15.0	48.2	114.8	1.9	6.0	1.5	16.3	249.9	16.7	1.1	27.3	10.1	1.3	34.5	43.8	1.29	6.02	3.36	0.18	0.69
LR1	Summer	32.3	153.1	836.3	217.4	869.4	3.5	22.9	6.0	8.3	101.5	12.6	47.8	28.8	7.3	72.2	3.9	283.7	2.67	30.42	3.71	0.08	0.75
LR3	Summer	2.5	32.4	2484.2	33.2	141.5	2.6	6.3	1.2	10.9	98.4	9.7	2.3	26.9	7.0	2225.9	1.2	2634.9	1.47	4.72	0.00	0.24	0.58
LR4	Summer	213.7	49.7	3263.3	82.7	186.2	5.0	1.2	1.1	7.3	58.9	8.3	6.7	32.3	17.0	34.6	1.8	60.6	0.63	20.58	8.50	0.03	0.13
LR5	Summer	2.5	28.6	94.3	45.3	151.5	6.6	0.7	1.1	10.2	100.5	11.7	1.7	26.9	5.3	123.4	54.8	6765.4	1.41	19.01	4.01	0.07	0.21
LR6	Summer	2.5	19.0	70.9	32.7	113.7	2.3	0.1	0.8	9.5	102.9	13.2	0.9	30.9	9.9	91.6	26.1	2392.0	0.61	7.12	11.57	0.08	0.26
NR1	Summer	535.3	102.4	3037.2	41.3	418.6	6.3	44.6	1.8	5.1	46.1	9.0	4.3	29.6	5.4	12.7	2.4	30.5	2.32	4.93	1.97	0.32	0.37
NR2	Summer	645.2	100.9	3062.9	75.6	538.8	7.9	50.2	2.7	7.3	69.3	11.8	5.1	28.7	5.3	10.8	2.9	31.7	1.75	7.94	1.25	0.18	0.22
NR3	Summer	35.0	31.9	215.9	26.1	272.5	5.6	11.1	1.2	6.7	95.2	18.9	3.8	29.4	5.6	15.3	27.1	414.6	1.71	4.52	0.49	0.27	0.30
NR4	Summer	22.3	72.1	480.8	15.7	394.8	5.4	24.5	1.9	7.1	69.6	12.1	14.2	28.2	5.9	49.1	12.6	619.8	1.76	4.84	0.64	0.27	0.33
NR5	Summer	50.9	93.6	83.5	62.9	474.4	7.3	2.4	2.6	7.1	71.7	11.2	18.2	32.1	5.3	6.4	58.2	369.8	3.58	12.30	7.45	0.23	0.49
YG1	Summer	20.2	50.8	109.8	39.5	443.6	7.0	29.1	2.2	6.2	112.2	19.3	5.8	30.3	5.2	16.4	54.9	899.7	1.35	5.69	11.85	0.19	0.19
YG2	Summer	68.6	125.9	94.3	60.6	998.2	6.5	18.2	4.9	5.9	101.2	17.5	40.3	30.0	4.1	5.0	49.2	245.6	6.07	27.07	6.96	0.18	0.93
YG3	Summer	100.5	55.2	135.8	182.1	263.5	8.5	2.6	1.5	7.0	70.3	10.6	8.4	26.9	1.8	7.0	31.1	217.3	2.22	7.73	10.65	0.22	0.26
MB1	Summer	347.6	884.3	797.6	38.4	1083.2	4.3	726.4	10.5	29.2	30.6	2.7	6.9	29.2	5.7	5.3	6.0	32.0	0.57	17.56	4.68	0.03	0.13
MB2	Summer	319.9	247.0	956.0	56.2	2676.2	5.1	223.0	30.8	18.2	321.3	23.9	7.8	29.6	6.1	7.0	5.9	41.3	1.36	6.45	2.20	0.17	0.27
MB3	Summer	2.5	70.9	39.3	18.0	910.8	3.8	22.1	5.8	8.3	211.9	28.4	41.5	29.0	7.7	50.7	77.1	3905.7	4.99	19.10	0.54	0.21	1.32
MB4	Summer	2.5	92.8	52.7	27.5	966.7	4.8	14.1	6.0	7.4	166.4	23.0	33.0	34.2	9.8	70.9	69.5	4924.8	4.05	23.82	6.88	0.15	0.85
LM1	Winter	14.8	5.8	677.1	35.1	140.0	1.8	2.8	0.4	2.9	156.1	53.1	1.7	6.2	11.7	106.7	3.0	320.0	0.53	0.00	0.52	0.87	0.29
LM2	Winter	12.0	1.9	15.0	70.3	63.5	1.8	0.7	0.4	3.5	545.5	75.0	0.4	5.7	13.0	15.7	25.2	395.4	0.30	0.29	0.00	0.51	0.16
LM3	Winter	77.3	10.8	475.4	80.9	169.0	2.0	1.5	0.7	4.6	159.4	34.6	5.0	6.7	15.8	15.9	4.3	68.2	0.96	0.90	0.00	0.52	0.47
LR1	Winter	890.6	30.3	4601.2	51.1	249.7	5.0	9.8	1.4	6.5	118.0	18.2	9.1	8.8	12.3	11.5	1.3	14.5	0.82	3.13	0.47	0.21	0.16
LR3	Winter	21.8	12.4	1203.8	31.4	160.5	5.3	2.9	0.9	6.4	185.4	28.5	1.0	8.8	9.4	125.5	5.0	629.9	0.89	8.41	3.61	0.10	0.17
LR4	Winter	2011.8	17.8	13731.0	55.2	157.5	6.7	0.9	0.6	4.5	88.5	19.6	5.6	12.2	19.0	15.1	0.6	8.6	0.94	2.28	0.00	0.29	0.14
LR5	Winter	14.8	26.2	326.1	77.5	268.9	37.9	4.6	1.9	8.4	186.9	22.7	4.2	12.3	9.5	60.5	109.5	6619.8	2.18	7.12	0.00	0.23	0.06
LR6	Winter	13.9	1.8	472.2	46.4	66.6	3.1	0.5	0.2	3.7	310.8	83.1	0.4	8.3	15.9	82.5	7.0	579.2	0.10	21.79	0.00	0.00	0.03
NR1	Winter	1717.6	74.3	4808.0	34.6	644.8	4.8	47.9	1.9	3.6	67.4	19.2	4.3	10.9	10.5	6.2	1.2	7.2	0.56	1.37	0.30	0.29	0.12
NR2	Winter	700.6	55.0	1793.6	43.1	757.5	5.5	35.3	1.9	3.0	90.6	30.4	5.1	9.9	11.4	5.8	3.5	20.2	0.98	2.26	0.03	0.30	0.18
NR3	Winter	50.8	16.6	49.3	15.1	321.5	4.1	5.6	0.7	2.6	109.3	42.7	1.8	10.1	12.2	2.8	74.5	208.9	0.31	0.72	0.00	0.30	0.08
NR4	Winter	39.2	38.7	406.8	25.8	356.6	4.7	13.8	1.2	3.9	79.8	20.4	5.8	11.0	11.2	24.4	12.7	309.0	0.98	1.68	0.00	0.37	0.21
NR5	Winter	1350.8	140.1	1484.3	55.5	902.9	6.5	41.6	4.2	5.4	76.8	14.2	57.8	11.6	16.1	2.5	4.9	12.4	1.59	7.01	0.58	0.18	0.24
YG1	Winter	53.8	57.3	71.4	56.7	513.4	7.7	53.6	2.1	4.8	95.6	19.8	7.5	12.2	9.5	5.3	69.9	367.9	0.81	4.72	1.38	0.15	0.11
YG2	Winter	32.9	87.6	84.7	34.1	735.4	8.1	26.5	3.2	5.1	94.7	18.6	20.1	15.1	7.6	8.0	79.2	631.7	2.23	7.37	0.76	0.23	0.28
YG3	Winter	50.7	83.5	73.3	30.1	514.3	9.2	9.7	2.4	5.5	73.8	13.6	12.5	12.3	11.3	4.5	104.1	469.6	3.97	13.22	1.87	0.23	0.43
MB1	Winter	15.9	81.7	125.9	45.3	1390.5	4.0	23.9	7.6	6.3	238.7	37.6	127.1	17.9	14.3	23.8	27.0	641.6	1.64	21.34	3.15	0.07	0.41
MB2	Winter	16.5	122.4	411.6	74.6	1362.4	4.1	14.6	4.8	4.1	100.7	24.6	121.4	18.2	11.2	65.1	9.9	644.4	1.53	16.28	5.19	0.09	0.37
MB3	Winter	119.7	47.3	1252.6	56.3	313.2	5.0	13.5	1.8	6.7	98.2	14.6	19.0	9.4	12.3	24.2	4.5	108.4	0.07	3.13	0.17	0.02	0.01
MB4	Winter	81.5	29.8	1082.2	51.6	237.1	5.3	3.1	1.1	5.3	93.6	17.6	12.7	10.2	21.5	30.8	5.5	168.9	0.56	3.39	0.73	0.14	0.10

Table 3.S2. Abundances for the prokaryotic groups assessed in the Brazos River watershed. Note that the proportional abundances of EUB and ARCH are of the DAPI counts, while the remaining groups are the proportion in relation to the EUB counts.

Site Name	Absolue Abundance Group										Proportional Abundance Group								
	DAPI	EUB	ARCH	ALF	BET	GAM	SRB	CF	HGC	LGC	EUB%	ARCH%	ALF%	BET%	GAM%	SRB%	CF%	HGC%	LGC%
LM1	1507493	801346	11424	27823	80983	20182	36937	46457	139371	37699	53.16%	0.77%	3.49%	10.96%	2.39%	3.98%	5.43%	14.18%	3.94%
LM2	1602997	928532	16450	148358	240358	100225	34729	42954	140742	41126	57.92%	0.87%	14.34%	22.60%	9.50%	3.03%	3.98%	12.84%	3.66%
LM3	1728202	1093949	11881	23762	535551	22848	47523	22848	189179	24676	63.30%	0.65%	2.02%	43.22%	1.97%	4.10%	1.98%	15.79%	2.06%
LR1	4603817	2458415	45695	159934	795101	169073	207914	139371	127947	61689	53.40%	0.84%	5.85%	28.46%	6.97%	7.39%	4.85%	6.52%	3.04%
LR3	2748582	1176658	22848	59404	662584	173643	57119	66258	203345	63974	42.81%	0.67%	4.26%	51.16%	11.44%	3.66%	4.47%	12.99%	4.45%
LR4	1526533	944220	14851	37699	483229	138229	45695	47980	29702	35414	61.85%	0.79%	3.09%	41.30%	9.76%	3.63%	3.80%	2.44%	3.01%
LR5	2297339	1545649	7997	11424	639736	29702	28560	60546	166788	13709	67.28%	0.34%	0.78%	45.48%	1.98%	1.89%	4.22%	11.76%	1.04%
LR6	1801543	1111542	6854	12566	492369	22848	18278	19421	38841	9139	61.70%	0.41%	1.21%	45.99%	2.27%	1.74%	1.79%	4.73%	1.08%
NR1	3582524	1617619	25133	91391	760068	126424	162980	94437	313014	93676	45.15%	0.50%	4.14%	42.16%	6.34%	7.10%	4.44%	10.31%	2.95%
NR2	5990674	3497987	27417	98245	1651891	331292	203345	180497	178212	86821	58.39%	0.45%	2.64%	44.86%	9.04%	5.42%	5.19%	4.08%	2.05%
NR3	5069911	2341892	54835	146225	1537652	317583	166788	127947	372418	114239	46.19%	1.05%	5.66%	59.44%	12.15%	6.83%	5.07%	11.91%	3.76%
NR4	6612132	3303781	47980	166788	1937487	381557	260464	198775	333577	86821	49.97%	0.74%	4.96%	60.42%	11.10%	8.58%	5.73%	9.30%	2.54%
NR5	5485739	3009046	22848	68543	1590202	146225	123378	75398	196490	63974	54.85%	0.38%	2.23%	50.05%	5.03%	4.15%	2.60%	5.97%	2.03%
YG1	3519058	1733889	117285	189636	130232	51788	94437	577286	534637	155365	49.27%	3.96%	11.79%	5.96%	2.60%	5.46%	24.97%	9.42%	2.87%
YG2	5195573	2513250	45695	89106	86821	79967	70828	191921	644306	155365	48.37%	0.80%	3.21%	3.44%	2.79%	2.68%	7.88%	9.34%	2.26%
YG3	1245658	682538	13789	91543	82252	45239	36785	42954	62603	19040	54.79%	2.41%	11.96%	9.01%	5.31%	4.35%	4.13%	5.93%	1.91%
MB1	26334290	12054461	173643	1005300	4660936	799670	1206360	1210930	1599341	548345	45.77%	0.75%	10.31%	46.50%	6.94%	11.18%	11.36%	9.66%	3.72%
MB2	23670245	10190086	365564	913909	3587093	936757	685432	365564	2216230	548345	43.05%	1.31%	9.98%	34.90%	11.42%	5.77%	3.15%	14.06%	3.62%
MB3	7197034	4469015	91391	169073	1041856	260464	164504	210199	274173	114239	62.10%	1.26%	4.26%	25.88%	7.03%	3.94%	4.86%	6.43%	2.87%
MB4	5401203	3002191	86821	233047	1014439	301590	278742	214769	329007	45695	55.58%	1.43%	6.39%	32.13%	9.42%	8.11%	6.65%	7.70%	1.22%

CHAPTER IV

DISCUSSION AND CONCLUSIONS

Riverine ecosystems are increasingly being studied as part of a larger landscape, as they are inextricably linked to the environmental conditions and activities that exist in their watersheds (Allan 2004). At the same time, they are important systems in their own right. On a per-area basis, the processing rates in freshwater aquatic systems are higher than terrestrial systems for carbon and many other nutrients (Cole et al. 2007, Tranvik et al. 2009). Additionally, freshwater ecosystems cover less than 1% of the earth's surface, yet are habitat for approximately 6% of all known species, and are under a disproportionate threat from anthropogenic effects (Abell et al. 2008). The study of large-scale spatial patterns in nutrient dynamics and species distributions provides many opportunities and challenges for understanding some of the core questions in ecosystem and community ecology, namely: Why do we see the existing patterns in productivity, community structure, diversity, etc.? (Currie 2007) The three studies included in this dissertation constitute an integration of datasets that are often analyzed separately, and rarely address spatial patterns on such a large scale. The combination of these studies provides insight into the underlying drivers and interactions that control riverine systems.

In Chapter 1, I assessed the degree to which relatively static measures of physiographic environmental conditions and patterns of land-use influence nutrient conditions in the Brazos River watershed. I found that in this system, which encompasses a broad range of environmental conditions, physiographic predictors

were more important in determining the patterns of nutrient conditions than land-use patterns. This is not to discount the influences that land-use has on aquatic systems: nitrogen inputs are influenced by the amount of urban and agricultural use in a watershed (Haggard et al. 2003, Dodds and Oakes 2006, Arango and Tank 2008), or changes in canopy cover around a stream can have strong effects on productivity and inorganic C cycling (Finlay 2003, Logue et al. 2004). The influence of physiographic parameters becomes apparent at large scales, or along steep environmental gradients (Malmqvist 2002, Goldstein et al. 2007), where it sets an overall baseline, that more proximate drivers of nutrient condition will interact with. From a management perspective, it is important to recognize that patterns of land use and physiography are not independent, and change on very long time-scales (King et al. 2005). Restoring a riparian buffer on a stream reach to reduce nitrogen input can have localized impact, but if the physiographic context or land-use in the larger area is such that nitrogen loading is broadly elevated, the restoration may have limited large-scale impact (Bernhardt and Palmer 2011). Thus, researchers and managers need to focus projects on the appropriate areas of a watershed and temper their expectations about the results of management programs so that they are aligned with the landscape context in which a river exists.

In Chapter 2 of this dissertation I applied a landscape scale perspective to questions of concordance between physicochemical conditions (largely the nutrients from Chapter 1 and habitat parameters), macroinvertebrate communities, and fish communities. Biotic communities are influenced by physicochemical conditions, as well as by predator-prey dynamics, competition, dispersal, and niche partitioning (Currie 2007). A recent topic in community ecology is understanding if the patterns of distribution in biotic communities is controlled largely by exogenous environmental influences, that are themselves spatially arranged (called induced spatial dependence) or whether factors such as dispersal ability or competition

(as types of endogenous spatial autocorrelation) are responsible for large-scale community patterns (Bahn and McGill 2007, Currie 2007, Peres-Neto and Legendre 2010). At the scale investigated in the Brazos River watershed, I found evidence that physicochemical conditions, macroinvertebrate communities, and fish communities were all responding to broad-scale environmental gradients. All three sets of data had similarly shaped correlograms, and when the effect of regional space was accounted for, only fish (putatively the group with the best dispersal ability), showed any pattern indicating autocorrelation between sites. The other major finding was that while there were significant patterns of concordance between taxa, the ability of one taxon to predict the other was relatively low. This has implications for managers, who use surrogate species or groups to assess overall ecosystem health (Padial et al. 2012). For the use of a surrogate species or group to be effective, there should be strong concordance and predictive ability between the surrogate and the large group it represents (Heino 2010, Padial et al. 2012). In the Brazos River these patterns of concordance, while significant and informative about the important gradients structuring the communities, were not strong enough for reliable prediction.

In Chapter 3 of this dissertation I used a landscape perspective to elucidate the relationship between patterns of bacterial function and community composition, and their relationships with physicochemical nutrient conditions. The processing and transformation of carbon compounds is one of the major ecosystem functions of bacteria (Cole et al. 2007). Understanding the patterns of how bacteria process carbon in sub-tropical regions also gives insight into changes that may happen in areas that will be affected by climate change, as the delivery of terrestrial organic matter is likely to change (Herron et al. 2009, Billings and Ballantyne 2013). I found that unlike many systems in more temperate regions, bacterial production and growth efficiency were not related. In temperate rivers, lakes, and estuaries,

there is commonly a positive relationship between bacterial production and growth efficiency, as production rates are typically variable, and respiration rates are typically stable (del Giorgio and Cole 1998, Maranger et al. 2005). It appears that the metabolic maintenance costs of bacteria are higher and more variable in this sub-tropical region. Additionally, it appears that both cell growth and maintenance are reliant on the use labile organic carbon sources, presumably from in-stream production. As in other studies, the link between bacterial community composition and function in the Brazos River watershed was weak (Langenheder et al. 2005).

Bacterial production was related to total community abundance, to a point, however sites with extremely high bacterial abundances did not have resulting high production rates, and none of the investigated bacterial groups were correlated with increases in bacterial production. The findings for the other measures of bacterial function are also equivocal. Bacterial communities have a large amount of functional redundancy, both within and between major groups, so at a coarse level of investigation, it is not surprising that these relationships would be weak (Langenheder et al. 2005, Comte and del Giorgio 2011). The final finding of this study was that there were shifts in the overall community composition in relation to nutrients and basin position of the sampling site. While a portion of this was driven by bacterial abundance, there were differences between how some bacteria responded to nutrient differences in the watershed. Most bacteria in the Brazos River watershed were correlated with particulate loading, β-proteobacteria were highest in areas with elevated NO_3^- concentrations and *Actinobacteria* were highest in areas with elevated SRP. While there were differences between β-proteobacteria and *Actinobacteria*, I did not find evidence for stronger competitive or exclusionary interactions between the groups (Ruiz-Gonzalez et al. 2013).

The series of studies in this dissertation highlight the utility of using a large-scale, landscape perspective when studying the ecology aquatic ecosystems.

By integrating studies on nutrients, macroinvertebrates, fish, and bacterial communities, I was able to identify common patterns among the multiple datasets. All four of the various response datasets indicate qualitatively similar patterns, with significantly different concentrations or communities between the upstream, northwest portions of the drainage basin and downstream, southeast portions of the drainage basin, indicating that there are large-scale environmental gradients influencing the nutrients and communities. Taken together, this provides evidence as to the importance of environmental gradients, functional zones, and the longitudinal patterns that occur in riverine systems (Vannote et al. 1980, Thorp et al. 2010). From a management or restoration perspective, it is important to identify the major controls on communities, so that efforts can go into areas with the largest potential benefit. Evidence is mounting that surrogate species or groups are something that should be considered carefully, at best (Padial et al. 2012). Additionally, riverine aquatic systems must be seen as part of the landscape, as impacts to the landscape, either through landscape modification or climate change will eventually impact the riverine system. For example, changes in bacterial function, either due to changes in nutrient delivery or processing rates will feed back into the macrobiotic communities through the alteration of nutrient availability to low trophic level communities that rely on bacterial processing of organic matter, potentially affecting humans through the ecosystem services that we rely on from riverine systems. Without better knowledge of what these changes may entail, it will be difficult to adapt management and restoration efforts and focus them on the highest priority areas.

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VITA

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